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(54) Title: SCHIZOPHRENIA RELATED GENES

(57) Abstract: There are provided isolated polynucleotide fragments for use in diagnosing and/or developing treatments for schizophrenia. Further provided is a method for diagnosing schizophrenia using one or more polynucleotides disclosed herein. Also provided is a method for screening a compound which regulates expression of a schizophrenia-related gene. Also provided is a chronic animal model of schizophrenia that mimics the functional deficits observed in patients and methods for producing the animal model comprising the administration of PCP to the animal.

# SCHIZOPHRENIA RELATED GENES

The present invention relates to the identification of genes postulated to be involved and/or associated with schizophrenia. The present invention also relates to the development of a chronic animal model which mimics functional deficits in schizophrenia and to the use of the model in drug screening and identification of genes/proteins associated with schizophrenia, as well as particular identified genes and their use in therapy/diagnosis of schizophrenia.

Schizophrenia is a devastating mental illness which affects 1% of the world population, the aetiology of which remains elusive. To date, there is a poor understanding of the genes involved and no chronic animal models of schizophrenia have been developed which imitate all the characteristics of the disease.

One of the goals of modern antipsychotic drug development is to produce a drug which is more effective in ameliorating the negative symptoms and cognitive deficits characteristic of schizophrenia than existing therapies. Although typical and atypical antipsychotic drugs, such as haloperidol and clozapine, are effective in attenuating the positive symptoms, they are ineffective (haloperidol) or minimally effective (clozapine) against the negative symptoms and cognitive dysfunction associated with the disease (Goldberg, T. et al). The development of improved antipsychotic drugs which will have superior action against

the negative symptoms and cognitive dysfunction has been severely hampered by the lack of knowledge of which genes are involved and/or associated with schizophrenia, or lack of an animal model which accurately models these symptoms.

Many putative models of schizophrenia have been described to date. These range from developmental models (Lillrank et al), social isolation (Jones, G.H. et al) or social interaction (Sams-Dodd, F. et al) models to pharmacological models (Snyder, S.H. et al). The major drawbacks of the present pharmacological models of schizophrenia are that they are based on acute administration of the drugs. The models involve administering the drug to produce the psychotic state, but in order to test the activity of antipsychotic drugs, they are administered before the animal is exposed to amphetamine or Phencyclidine (PCP). This would be tantamount to administering an antipsychotic drug to a patient before the onset of schizophrenia. The models also do not account for the fact that antipsychotic treatment can take up to a month to have beneficial effects against the disease. Thus, the current models of schizophrenia fail to accurately mimic the clinical profile of the disease.

Moreover, little is known about the genes, or more specifically any alteration of expression/mutation of genes in a patient suffering from schizophrenia.

It is therefore amongst the objects of the present

invention to obviate and/or mitigate at least one of the aforementioned disadvantages.

The present invention is based in part on the development of a chronic animal model of schizophrenia using the drug phencyclidine (PCP) and the use of this model to identify genes thought to be involved and/or associated with schizophrenia. Although PCP has been known for many years to produce schizophrenic-like symptoms in man and also to worsen the psychotic state in schizophrenics (Allen, R.M. et al), it has hitherto not been used to develop a chronic animal model of schizophrenia that mimics the functional deficits observed in patients.

The present invention is also based in part on the elucidation of genes which are differentially expressed in the blood of schizophrenic patients.

Thus, according to a first aspect, there are provided isolated polynucleotide fragments for use in diagnosing and/or developing treatments for schizophrenia. The isolated polynucleotide fragments are shown in the attached Figures 1, 2, 3, 4, 5a, 6a, 6c, 6e, 7a, 8a, 9a, 9c and 10a. The inventors have presently identified 10 genes which have been observed to be differentially expressed in the animal model disclosed herein or in blood samples from schizophrenic patients. The genes have been designated YSG1-10. The YSG3 (Figure 1: SEQ ID No. 1), YSG4 (Figure 2: SEQ ID No. 2), YSG6 (Figure 3: SEQ ID No. 3) and YSG9

(Figure 4: SEQ ID No. 4) are shown to be novel sequences based on database screening. The remaining sequences are known genes not however previously being associated with schizophrenia; YSG1 (Figure 5a: SEQ ID No. 5) relates to phosphodiesterase 1α; YSG2 (Figures 6a, 6c, 6e: SEQ ID Nos. 7, 9 & 11, respectively) relates to calcium-independent alpha-latrotoxin receptor (CIRL 1, 2 & 3); YSG5 (Figure 7a: SEQ ID No. 13) relates to epithelial discoidin domain receptor 1, trkE; YSG7 (Figure 8a: SEQ ID No. 15) relates to netrin receptor UNC5H1; YSG8 (Figures 9a, 9c: SEQ ID Nos. 17 & 19, respectively) relates to synapsins 1A and 1B; and YSG10 (Figure 10a: SED ID No. 21) relates to TNFα.

Thus the present invention provides a polynucleotide having DNA sequence represented by SEQ ID No. 1; a polynucleotide having DNA sequence represented by SEQ ID No. 2; a polynucleotide having DNA sequence represented by SEQ ID No. 3; or a polynucleotide having DNA sequence represented by SEQ ID No. 4.

The present invention also provides a method for diagnosing schizophrenia which comprises using one or more polynucleotides selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 (PDE1 α), SEQ ID No. 7, 9 & 11 (CIRL 1,2 & 3), SEQ ID NO. 13 (trkE), SEQ ID No. 15 (netrin receptor), SEQ ID. No. 17 & 19 (synapsin 1A/1B) and SEQ ID No. 21 (TNFα) as indicator(s).

The above described polynucleotide fragments have been

discovered to be differentially expressed in a chronic animal model as described herein or in the blood of schizophrenic patients and are postulated therefore to be putatively involved and/or associated with schizophrenia.

"Polynucleotide fragment" as used herein refers to a polymeric form of nucleotides of any length, both to ribonucleic acid sequences and to deoxyribonucleic acid sequences. In principle, this term refers to the primary structure of the molecule. Thus, the term includes double stranded and single stranded DNA, as well as double and single stranded RNA, and modifications thereof.

In a further aspect the present invention provides polynucleotide fragments encoding polypeptides for use in diagnosing and/or developing treatments for schizophrenia.

In particular the polypeptides are shown in Figures 5b, 6b, 6d, 6f, 7b, 8b, 9b, 9d and 10b, relating to SEQ ID Nos. 6, 8, 10, 12, 14, 16, 18, 20 & 22.

In general, the term "polypeptide" refers to a molecular chain of amino acids with a biological activity. It does not refer to a specific length of the product, and if required can be modified in vivo and/or in vitro, for example by glycosylation, myristoylation, amidation, carboxylation or phosphorylation; thus inter alia peptides, oligopeptides and proteins are included. The polypeptides disclosed herein may be obtained by synthetic or recombinant techniques known in the art.

Thus, the term extends to cover for example

polypeptides obtainable from various transcripts and splice variants of these transcripts from a particular gene.

It will be understood that for the polynucleotide fragments and polypeptide sequences presented herein, natural variations can exist between individuals. These variations may be demonstrated by nucleotide and/or amino acid differences in the overall sequence or by deletions, substitutions, insertions or inversions of nucleotides or amino acids in said sequences.

As is well known in the art, the degeneracy of the genetic code permits substitution of bases in a codon resulting in another codon for the same amino acid. Consequently, it is clear that any such derivative nucleotide sequence based on the sequences disclosed herein are also included in the scope of the present invention.

Thus, the present invention further includes nucleotide and/or polypeptide sequences having at least 80%, particularly at least 90%, and especially at least 95% homology or similarity with the sequences shown in the attached Figures.

The present invention also includes nucleotide sequences similar to the polynucleotide sequences disclosed herein. It is understood that similar sequences include sequences which remain hybridised to the polynucleotide sequences of the present invention under stringent conditions. Typically a test similar sequence and a polynucleotide sequence of the present invention are

allowed to hybridise for a specified period of time generally at a temperature of between 50 and 70°C in double strength SSC (2 x NaCl 17.5g/l and sodium citrate (SC) at 8.8 g/l) buffered saline containing 0.1% sodium dodecyl sulphate (SDS) followed by rinsing of the support at the same temperature but with a buffer having a reduced SSC concentration. Depending upon the degree of similarity of the sequences, such reduced concentration buffers are typically single strength SSC containing 0.1% SDS, half strength SSC containing 0.1% SDS and one tenth strength SSC containing 0.1% SDS. Sequences having the greatest degree of similarity are those the hybridisation of which is least affected by washing in buffers of reduced concentration. It is most preferred that the similar and inventive sequences are so familiar that the hybridisation between them is substantially unaffected by washing or incubation in one tenth strength SSC containing 0.1% SDS.

Furthermore, fragments derived from the polynucleotide fragments depicted in the Figures may be used.

Moreover, fragments derived from the encoded polypeptides are also encompassed by the present invention.

All such modifications mentioned above resulting in such derivatives of the polypeptides are covered by the present invention so long as the characteristic polypeptide properties remain substantially unaffected in essence.

The information presented herein can be used to genetically manipulate the sequences or derivatives

thereof, for example to clone the sequences by recombinant DNA techniques generally known in the art. Cloning of homologous sequences from other species of mammal, and in particular humans, may be performed with the information disclosed herein by widely known techniques; for example, oligonucleotides may be designed to a consensus region and/or functional domains of the sequences shown in the Figures and such oligonucleotides, and/or the polymerase chain reaction products generated using these oligonucleotide primers, can be used as probes for cloning homologous sequences from other organisms, for example by polymerase chain reaction or by hybridisation.

The polynucleotide fragments of the present invention may be linked to expression control sequences. Such control sequences may comprise promoters, operators, inducers, ribosome binding sites etc. Suitable control sequences for a given host may be selected by those of ordinary skill in the art.

A nucleotide sequence according to the present invention can be ligated to various expression-controlling DNA sequences, resulting in a so-called recombinant nucleic acid molecule. Thus the present invention also includes an expression vector comprising an expressible nucleotide sequence. Said recombinant nucleic acid molecule can then be used for transformation of a suitable host.

Such recombinant nucleic acid molecules are preferably derived from for example, plasmids, or from nucleic acid

sequences present in bacteriophages or viruses and are termed vector molecules.

Specific vectors which can be used to clone nucleotide sequences according to the invention are known in the art (eg. Rodriguez RL and DT Denhardt, editors, Vectors: A survey of molecular cloning vectors and their uses, Butterworths, 1988).

The methods to be used for the construction of a recombinant nucleic acid molecule according to the invention are known to those of ordinary skill in the art and are inter alia set forth in Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, 1989.

The present invention also relates to a transformed cell comprising the polynucleotide fragments of the present invention, in expressible form, if appropriate. "Transformation", as used herein, refers to the introduction of a heterologous nucleic acid sequence into a host cell *in vivo*, *ex vivo* or *in vitro* irrespective of the method used, for example, by calcium phosphate co-precipitation, direct uptake, electroporation or transduction.

The heterologous nucleic acid sequence may be maintained through autonomous replication or alternatively may be integrated into the host's genome. The recombinant DNA molecules preferably are provided with appropriate control sequences, compatible with the designated host

which can regulate the expression of the inserted nucleic acid sequence.

The most widely used hosts for expression of recombinant nucleic acid molecules are bacteria, yeast, insect cells and mammalian cells. Each system has advantages and disadvantages in terms of the vector used, potential ease of production and purification of a recombinant polypeptide and authenticity of product in terms of tertiary structure, glycosylation state, biological activity and stability and will be a matter of choice for the skilled addressee.

In addition to expressing the polynucleotide fragments of the present invention, in certain circumstances, it is advantageous to substantially prevent or reduce the expression or activity of the polynucleotide fragments in a cell or host. Thus, according to a further aspect of the invention, there is provided an antisense nucleotide fragment complementary to a polynucleotide fragment or subfragment of the present invention. Included in the scope of "antisense nucleotide fragment" is the use of synthetic oligonucleotide sequences, or of equivalent chemical entities known to those skilled in the art, for example, peptide nucleic acids. Also provided is a nucleotide fragment comprising a nucleotide sequence which, when transcribed by the cell, produces such an antisense fragment. Typically antisense RNA fragments will be provided which bind to complementary mRNA fragments to form

RNA double helices, allowing RNAse H to cleave the molecule and rendering it incapable of being translated by the cell into polypeptides.

A further aspect of the present invention provides antibodies specific to the polypeptides of the present invention or epitopes thereof. Production and purification of antibodies specific to an antigen is a matter of ordinary skill, and the methods to be used are clear to those skilled in the art. The term antibodies can include, but is not limited to polyclonal antibodies, monoclonal antibodies (mAbs), humanised or chimeric antibodies, single chain antibodies, Fab fragments, $F(ab')_2$ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, and epitope binding fragments of any of the above. Such antibodies may be used in modulating the expression or activity of the particular polypeptide, or in detecting said polypeptide in vivo or in vitro.

The present invention further provides a recombinant or synthetic polypeptide for the manufacture of reagents for use as therapeutic agents in the treatment of schizophrenia. In particular, the invention provides pharmaceutical compositions comprising the recombinant or synthetic polypeptide together with a pharmaceutically acceptable carrier therefor.

The present invention also relates to methods for prognostic and/or diagnostic evaluation of schizophrenia

and/or for the identification of subjects who are predisposed to schizophrenia, for example by examination of allelic variation by determination of the expression or sequence of the genes identified herein in an individual. Furthermore, the invention provides methods for evaluating the efficacy of drugs for such disorders, and monitoring the progress of patients involved in clinical trials for the treatment of such disorders.

Thus the invention further provides methods for the identification of compounds which modulate the expression of the polynucleotide fragments and/or the activity of polypeptide sequences identified herein. Such identified compounds may be used in the treatment of schizophrenia.

Thus there is provided a method for screening a compound which regulates expression of a schizophrenia-related gene(s), which comprises:

(a) bringing a test compound into contact with transformed cell which enables expression of a gene selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 (PDEI α), SEQ ID No. 7, 9 & 11 (CIRL 1, 2 & 3), SEQ ID No. 13 (trkE), SEQ ID No. 15 (netrin receptor), SEQ ID No. 17 & 19 (Synapsin 1A/AB) and SEQ ID No. 21 (TNFα),

(b) detecting an expression of schizophrenia-relating factor in said cell, and

(c) selecting a compound which promotes or suppresses an induction of the schizophrenia-relating factor in

comparison with a control (vehicle).

There is also provided a method for measuring an anti-schizophrenic effects of a compound using the animal model of the present invention, which comprises:

(a) measuring local cerebral glucose utilisation (LCGU), or detecting an expression level of one or more genes represented by the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 (PDE 1α), SEQ ID No. 7, 9 & 11 (CIRL 1, 2 & 3), SEQ ID No. 13 (trkE), SEQ ID No. 15 (netrin receptor), SEQ ID No. 17 & 19 (synapsin 1A/1B) and SEQ ID No. 21 (TNFα), and

(b) comparing with a control group.

The biological function of the genes identified herein can be more directly assessed by utilizing relevant *in vivo* and *in vitro* systems. *In vivo* systems can include, but are not limited to, animal systems which naturally exhibit the symptoms of schizophrenia, or ones which have been engineered to exhibit such symptoms, as for example the model described herein. Further, such systems can include, but are not limited to transgenic animal systems. *In vivo* systems can include, but are not limited to, cell-based systems comprising the identified gene/polypeptide expressing cell types. The cells can be wild type cells, or can be non-wild type cells containing modifications known or suspected of contributing to schizophrenia.

In further characterising the biological function of said identified gene(s), the expression of said identified

gene(s) can be modulated within the *in vivo* and/or *in vitro* systems, i.e. either overexpressed or underexpressed in, for example, transgenic animals and/or cell lines, and its subsequent effect on the system can then be assayed. Alternatively, the activity of the product of the identified gene can be modulated by either increasing or decreasing the level of activity in the *in vivo* and/or *in vitro* system of interest, and its subsequent effect then assayed.

The information obtained through such characterisations can suggest relevant methods for the treatment or control of schizophrenia. For example, relevant treatment can include a modulation of gene expression and/or gene product activity. Characterisation procedures such as those described herein can indicate whether such modulation should be positive or negative. As used herein, "positive modulation" refers to an increase in gene expression or activity of the gene or gene product of interest. "Negative modulation", as used herein, refers to a decrease in gene expression or activity.

*In vitro* systems can be designed to identify compounds capable of binding said identified gene(s) products of the invention. Compounds identified can be useful, for example, in modulating the activity of wild type and/or mutant gene(s) products, can be useful in elaborating the biological function of said identified gene(s) products, or

can disrupt normal identified gene(s) product interactions.

In another aspect the present invention provides a chronic animal model of schizophrenia that mimics the functional deficits observed in patients wherein the animal model has been developed by the addition of PCP to an animal.

In a further aspect the present invention provides a method for developing a chronic animal model of schizophrenia, said method comprising the steps of:

a) administering PCP to an animal in order to induce a psychotic state in the animal representative of the onset of schizophrenia in humans; and

b) further administrating of PCP in order to maintain the PCP-induced psychotic state in the animal, over a period of time, to mimic a chronic state of schizophrenia in the animal.

The present invention also relates to an animal model produced by the method(s) of the present invention.

The animals of the present invention may be any suitable non-human animal. Typically the animal is a rat, mouse, guinea pig, rabbit or the like.

As mentioned above the present invention relates to the development of a chronic animal model. It is understood that the term chronic relates to a disease which is deep-seated or long-continued as opposed to an acute or rapidly developed disease.

The present inventors have developed a chronic

treatment paradigm which comprises two phases. The initial phase involves a period of treatment with PCP which was hypothesised would induce a psychotic state within the animal such as a rat, representing the onset of the disease in humans. The second phase concerns the maintenance of this PCP-induced psychotic state over a time period which would allow the incorporation of chronic antipsychotic therapy, relating to the therapeutic delay in antipsychotic efficacy observed in humans. The observation of a psychotic state may be measured in a number of ways. However, the measurement of the "psychotic state" was determined by the present inventors as PCP-induced hypofrontality which is observed in similar human imaging studies and is correlated to the negative symptoms and cognitive dysfunction associated with chronic schizophrenia (Wolkin, A. et al).

The initial administration of PCP to animal must be sufficient to induce a psychotic state and further administration of PCP must be sufficient to maintain the PCP-induced psychotic state. The present inventors have observed that an initial amount of PCP required to induce a psychotic state may be insufficient to maintain and mimic a chronic state of schizophrenia in the animal.

It has been previously observed that a level of 0.86 $mgkg^{-1}$ is sufficient to induce an acute state of schizophrenia in an animal model, but the present inventors have found that this is insufficient to maintain and induce

a chronic state. The present inventors have used a level of 2.58 $mgkg^{-1}$ to maintain and induce a chronic state of schizophrenia in a rat model. Thus, the present invention provides a method for developing a chronic animal model of schizophrenia which includes administering a level of 1 to 5 $mgkg^{-1}$ PCP, for example, a level of 2 to 4 $mgkg^{-1}$, such as, a level of 2.58 $mgkg^{-1}$ to an animal to induce a chronic state of schizophrenia.

The effects of this PCP treatment paradigm on dopamine utilisation within selected brain areas was also investigated by HPLC analysis. The levels of dopamine metabolites within plasma and CSF of schizophrenic patients has been established and it has been found that chronic schizophrenics have lower levels of both homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) compared to controls (Heritch, A.J). This implies that there is decreased turnover of dopamine within the schizophrenic brain.

For a working animal model of a disease to be valid there are certain underlying criteria which are fundamental and which must be taken into consideration. The first criteria, construct validity, is defined as the ability of the model to mimic the underlying neurobiological abnormalities which are core characteristics of the disease. This is difficult to emulate for schizophrenia, since the aetiology of the disease is far from clear. The second criteria, face validity, is defined as the model

must produce symptomatologies that resemble those characteristically observed in the disease. The third criteria, predictive validity, is defined as drugs which have established action against a disease must restore parameters in the animal model to normal, whereas other classes of drugs should be inactive.

The chronic PCP model described here satisfies these criteria to an impressive degree. The model uses a drug which is known to produce effects in humans which are analogous to those observed in schizophrenia.

Although the psychotic state may not be triggered by the same mechanism it is likely, from the evidence produced, that the psychosis is being mediated by the same systems which are implicated in the dysfunction associated with schizophrenia, such as the glutamatergic (Tamminga, C.) and dopaminergic (Angrist, B. et al) systems. The model also shows altered function in specific neural circuits, the corticothalamic and temporolimbic circuits, which have been shown to be abnormal in schizophrenia (Swerdlow, N.R. et al and Weinberger, D.R). The model also has face validity, with metabolic hypofunction, and changes in receptor binding being observed with this model and in schizophrenia. The predictive validity of the model is more difficult to evaluate, although the lack of reversibility of the prefrontal cortex hypofunction mirrors the clinical observations. However, the attenuation of the hypofunction within the auditory system by known

antipsychotic drugs suggest that this model does have predictive validity.

The model was also studied for parvalbumin expression which has been shown to be decreased in post mortem tissue of schizophrenic subjects. Parvalbumin expression in the model was also reduced in the prefrontal cortex, as observed in schizophrenic subjects. The model thus reproduces an established pattern of brain dysfunction associated with schizophrenia. This observation may have utility in developing novel antipsychotic drugs.

The model finds particular application in the screening of new drugs for treating schizophrenia. Thus, test drugs may be administered to the animal model and their effect on psychotic conditions observed. The present invention therefore also relates to new anti-schizophrenic drugs identified using the animal model of the present invention.

The model also allows the detection of genes, the expression of which is altered, as compared to a "normal" animal. A "normal" animal is one which has not been induced to the chronic psychotic state and which exhibits normal behaviours.

Genes identified in this manner may be associated with the schizophrenic state. Therefore identification of such genes allows their study and/or development of therapies designed to return expression to normal.

The present invention will now be further described by

way of non-limiting example and with reference to the attached Figures (where CLO indicated clozapine and HAL indicates haloperidol) which show:

Figures 1 - 4 show the nucleotide sequence of four sequences observed to be differentially expressed in the brain of the rat model of the present invention;

Figure 5a shows the nucleotide sequence and Figure 5b shows the polypeptide sequence of phosphodiesterase 1α which have been observed to be differentially expressed in the brain of the rat model of the present invention;

Figures 6a, 6c and 6e show the nucleotide sequences and Figures 6b, 6d and 6f show the polypeptide sequences of calcium-independent alpha-latrotoxin receptor which have been observed to be differentially expressed in the brain of the rat model of the present invention;

Figure 7a shows the nucleotide sequence and Figure 7b shows the polypeptide sequence of epithelial discoidin domain receptor, trkE, which has been observed to be differentially expressed in the blood of schizophrenic patients as compared to normal controls;

Figure 8a shows the nucleotide sequence and Figure 8b shows the polypeptide sequence of netrin receptor which has been observed to be differentially expressed in the brain of the rat model of the present invention;

Figures 9a and 9c show the nucleotide sequence and Figures 9b and 9d show the polypeptide sequence of synapsins 1A and 1B which have been observed to be

differentially expressed in the brain of the rat model of the present invention;

Figure 10a shows the nucleotide sequence and Figure 10b shows the polypeptide sequence of YSG9 (Seq ID No. 19) which has been observed to be differentially expressed in the brains of schizophrenic patients and PCP-treated rats as compared to normal controls;

Figure 11 is a histogram showing the relative expression levels of genes in human blood samples;

Figure 12 shows parvalbumin expression in brain tissue of the animal model of the present invention;

Figure 13 illustrates the level of CIRL1 mRNA present in the BA11 region of schizophrenic (grey dashed line, n=6) and control (black, n=8) post-mortem tissue. TCTCCTGGCTGTGCCTGGAGGGC and GGCTTGAGCACAGATCAGCTTCGG were the primer sequences used to amplify this product.

Figure 14 illustrates the level of CIRL1 mRNA present in the prefrontal cortex of rat brain following chronic PCP and antipsychotic treatment (n=12 for all groups apart from veh-veh where n=11). TCTCCTGGCTGTGCCTAGAGGGC and GGCTTGAGCACGGATGAGCTTCGG were the primer sequences used to amplify this product.

Figure 15 illustrates the level of CIRL2 variant AB mRNA present in the prefrontal cortex of rat brain following chronic PCP and antipsychotic treatment (n=12 for all groups apart from veh-veh where n=11). GGAAAACATTAAGTCTTGGGTG and GTGAATGTCCTTGATTAAGGGT were the

primer sequences used to amplify this product.

Figure 15 illustrates the level of CIRL3 variant AA mRNA present in the prefrontal cortex of rat brain following chronic PCP and antipsychotic treatment (n=12 for all groups apart from veh-veh where n=11). GTAGTTCATGCTTTCAGCCGT and AGAAGCCCCTCTCTGTTGAG were the primer sequences used to amplify this product.

Figure 17 illustrates the expression profile of TNFα 2 and 24hrs after a single i.p. injection of PCP at 2mg/kg (N=4 for all treatment groups). AGGAGGAGAAGTTCCCAAATG and TTGTCCCTTGAAGAGAACCTG were the primer sequences used to amplify this product.

Figure 18 illustrates the levels of TNFα in rat prefrontal cortex following chronic PCP and antipsychotic treatment (n=12 for all groups apart from veh-veh where n=11). AGGAGGAGAAGTTCCCAAATG and TTGTCCCTTGAAGAGAACCTG were the primer sequences used to amplify this product.

Figure 19 illustrates the levels of TNFα in postmortem orbital frontal cortex of schizophrenics (n=4) and controls (n=5). GGTAGGAGACGGCGATGC and CAGGCAGTCAGATCATCTTC were the primer sequences used to amplify this product.

## Example 1 - Development of rat model

An initial treatment period of intraperitoneal (i.p.) injections once daily for 5 days was carried out, followed by a maintenance schedule of i.p. injections three times weekly (every 60 hours) for a further 21 days.

Intermittent exposure to PCP during the maintenance phase of the model was favoured due to the long half life of the drug within brain tissue (Misra, A.L. et al). The doses of PCP chosen represented the selective blockade of the NMDA channel (0.86 $mgkg^{-1}$) and a dose (2.58 $mgkg^{-1}$) which is pharmacologically less selective but less that the $ED_{50}$ for PCP-induced cell death. As a comparison to the present model, the inventors also investigated the effect of previously published subchronic treatment with PCP (Jentsch, J.D. et al) using quantitative C-2-deoxyglucose autoradiography (Sokoloff, L.).

Local cerebral glucose utilisation (LCGU) was measured using an adaptation of the original method for freely moving rats (Crane, A.M. et al) 72 hours after the initial induction phase (day 8) and 72 hours after the induction phase followed by the maintenance phase (day 29). LCGU was measured 72 hours after the last exposure to PCP so the effects of PCP on LCGU would be independent of the acute effects of the drug. Table 1 shows the results from the induction and maintenance phases of the model. The dose of 2.58 $mgkg^{-1}$ PCP induced a metabolic hypofunction which was evident after both phases of the model within the medial orbital cortex, the prelimbic cortex, the auditory pathway and the reticular nucleus of the thalamus. The metabolic hypofunction produced by the lower dose of PCP (0.86 $mgkg^{-1}$) within these areas during the initial phase of the model

was not, however, maintained by the subsequent second phase of the model. Thus, using a dose of 2.59 $mgkg^{-1}$ the inventors had established a novel treatment paradigm which mimics the findings of human imaging studies in schizophrenic patients. In comparison, the previously published subchronic treatment (Jentsch et al) with PCP (5 $mgkg^{-1}$ twice daily for seven days) did not produce any significant effect on LCGU within any brain area (data not shown).

In summary, the data provided from the animal studies utilising this chronic PCP model mimic those previously published from human imaging studies and post mortem studies of schizophrenic brain tissue from schizophrenic patients. Since human imaging studies have correlated the prefrontal hypofunction to the negative symptoms of schizophrenia (Wolkin, A. et al, 1992) and abnormalities of the temperolimbic system (including the auditory system and hippocampus) and thalamus to the positive symptoms of schizophrenia (Tamminga, C.A. et al, 1992), it can be proposed that this model mimics both the positive and negative symptoms of the disease. As such, this model has superior construct, face and predictive validity than existing animal models of schizophrenia.

Table 1: Induction and maintenance of PCP-induced hypofunction

| | LCGU (µmol/100g/min) | | | | | |
|---|---|---|---|---|---|---|
| | | day 8 | | | day 29 | |
| | vehicle | 0.86 PCP | 2.58 PCP | vehicle | 0.86 PCP | 2.58 PCP |
| *Prefrontal Cortex* | | | | | | |
| mO layer | 131±4 | 110±2* | 105±4* | 125±4 | 122±3 | 108±5* |
| mO layers II & III | 137±4 | 127±2 | 127±5 | 147±3 | 135±4 | 124±5 |
| mO layers V & VI | 140±1 | 129±5* | 115±1* | 137±2 | 136±3 | 111±3* |
| PrL layer I | 134±2 | 132±6 | 104±2* | 135±1 | 133±2 | 107±4* |
| PrL layers II & III | 154±3 | 150±7 | 133±2* | 152±2 | 149±2 | 116±1* |
| PrL layers V & VI | 114±3 | 115±3 | 96±3* | 114±2 | 112±3 | 89±2* |
| *Thalamus* | | | | | | |
| Rt | 116±2 | 106±2 | 86±2* | 118±4 | 108±4 | 89±2* |
| MD | 114±4 | 112±4 | 115±4 | 121±6 | 122±5 | 116±3 |

| | LCGU (µmol/100g/min) | | | | | |
|---|---|---|---|---|---|---|
| | | day 8 | | | day 29 | |
| | vehicle | 0.86 PCP | 2.58 PCP | vehicle | 0.86 PCP | 2.58 PCP |
| *Auditory System* | | | | | | |
| Au layer I | 159±11 | 127±2 | 135±3* | 158±13 | 167±14 | 137±5* |
| Au layers II,III&IV | 171±13 | 152±3 | 166±5 | 184±8 | 189±12 | 162±6 |
| Au layers V & VI | 122±8 | 114±4 | 120±4 | 128±9 | 137±10 | 115±3 |
| AuD layer I | 155±12 | 126±3* | 135±3* | 167±9 | 159±11 | 130±7* |
| AuD layers II,III&IV | 178±14 | 151±4* | 151±4* | 189±9 | 178±10 | 146±7* |
| AuD layers V & VI | 127±7 | 106±2* | 104±2* | 133±7 | 128±9 | 101±4* |
| DLL | 116±7 | 91±4* | 83±4* | 118±7 | 116±8 | 96±5* |
| VLL | 125±9 | 98±5* | 93±3* | 121±6 | 118±13 | 99±4* |
| cochlear nucleus | 126±4 | 107±3* | 98±3* | 124±3 | 117±2 | 94±4* |

Table 1: All data expressed as mean LCGU (µmol/100g/min) ± SEM (n=5-6). Statistical analysis carried out using individual one-way ANOVA for each discrete brain region followed by Fisher's least significant difference post hoc test where appropriate, with statistical significance defined as $p < 0.05$. * $p<0.05$ compared to controls. Day 8 data represents LCGU measured 72 hours

following the last exposure to PCP after 5 days i.p. injections once daily of 0.86 or 2.58 $mgkg^{-1}$ PCP or vehicle (sterile saline). Day 29 data represents LCGU measured 72 hours following the last exposure to PCP after i.p. injections once daily (day 1-5) and once daily on days 8, 10, 12, 15, 17, 19, 22, 24 and 26 of 0.86 or 2.58 $mgkg^{-1}$ PCP or vehicle (sterile saline).

Abbreviations: mO, medial orbital cortex; PrL, prelimibic cortex; Rt reticular nucleus of the thalamus; MD, mediodorsal nucleus of the thalamus; Au, primary auditory cortex; AuD, dorsal nucleus of the secondary auditory cortex; DLL & VLL, dorsal nucleus and ventral nucleus of the lateral lemniscus.

**Example 2 - Testing of rat model**

In order to establish the effect of antipsychotic drugs in the model, a second study was then carried out using a dose of 2.58 $mgkg^{-1}$ PCP which produced a metabolic hypofunction in the first studies, combined with antipsychotic therapy. The antipsychotic drugs were administered via osmotic minipumps for 21 days in order to maintain constant plasma concentrations of the drugs, which mirrored therapeutic plasma levels of the drugs in humans.

Table 2 shows the effect of haloperidol and clozapine alone and in conjunction with PCP treatment compared to

vehicle treated rats. Within the medial orbital cortex, the prelimbic cortex, the CA1 region of the hippocampus and the reticular nucleus of the thalamus, a metabolic hypofunction was again observed after treatment with PCP compared to controls. Clozapine and haloperidol also produced a metabolic hypofunction within these areas and failed to modulate the hypofunction produced by PCP. Within the auditory system, the dorsal nucleus of the secondary auditory cortex, dorsal and lateral nucleic of the lateral lemniscus and the cochlear nucleus, PCP again induced a metabolic hypofunction. However, within these regions, clozapine and haloperidol did not produce a significant hypofunction by themselves, but reversed the PCP-induced hypofunction when used in conjunction with the PCP. The inability of haloperidol and clozapine to modulate the hypofrontality is consistent with data from clinical studies and also the theory that this hypofrontality is associated with the negative symptoms and cognitive dysfunction of schizophrenia. The effect of antipsychotics on the positive symptoms is less well studied regarding imaging studies. There is no published evidence to date regarding the effect of haloperidol and clozapine within the temporal lobe structures (hippocampus and auditory cortex).

However, the ability of both antipsychotics to reverse the decreased glucose utilisation within the auditory system (auditory cortex, lateral lemniscus and the cochlear

nucleus) is consistent with the clinical evidence that both typical and atypical antipsychotics can improve ratings of positive symptoms of schizophrenia.

In order to further validate this chronic PCP model, the effects of this treatment paradigm on 5-$HT_{2A}$ receptors within the prefrontal cortex was investigated. Chronic PCP treatment produced a significant decrease in 5-$HT_{2A}$ receptors in layer II & III (controls 158±6, PCP 139±4 $fmolmg^{-1}$) and layers V & VI (controls 82 ±4, PCP 69±3 $fmolmg^{-1}$). This is entirely consistent with post mortem studies of 5-$HT_{2A}$ receptor binding from schizophrenic patents (Laurelle, M. et al).

In order to validate further this chronic PCP model the effect of this treatment paradigm on parvalbumin mRNA expression was investigated. A decrease in parvalbumin mRNA was observed after chronic PCP treatment within the prelimbic region of the prefrontal cortex (controls 0.0717±0.0011, PCP 0.0536±0.0023 relative optical density (ROD)). This PCP-induced decrease was reversed by clozapine (0.0693±0.0050 ROD) but not by haloperidol (0.0557±0.0022 ROD). PCP produced a significant decrease in parvalbumin mRNA within the ventral reticular nucleus of the thalamus (controls 0.6416±0.0122, PCP 0.5032±0.0194 ROD) which was reversed by both clozapine (0.6354±0.0173 ROD) and haloperidol (0.06199±0.0137) (see Figure 12). This decrease in parvalbumin expression is in agreement with studies of

schizophrenic post mortem tissue within the prefrontal cortex (Beasley & Reynolds, 1997) and anterior thalamus (Danos et al, 1998). The ability of clozapine but not haloperidol to reverse the decrease in parvalbumin expression in the prefrontal cortex is consistent with its ability to alleviate the cognitive deficits/negative symptoms in schizophrenia. Thus, reversal of parvalbumin deficits may be a useful marker for detecting atypical antipsychotic activity.

Methods

5-$HT_{2A}$ receptor binding: Sections from the level of the prefrontal cortex were preincubated for two consecutive washes at room temperature in 50mM Tris HCl buffer pH 7.4 to remove endogenous ligand. Total binding was defined using 0.71 nM (Wolkin, A. et al) $^3$H-ketanserin in the presence of 1µM prazozin and 1µM tetrabenazine (to block non 5-$HT_{2A}$ binding). Non-specific binding was defined using 50nM spiperone. Sections were incubated with the appropriate ligand solution for 1 hour at room temperature then washed twice for 10 minutes in ice cold buffer before being rinsed in ice cold water and rapidly air dried. The sections were then exposed to film (Biomax MR, Kodak) with previously calibrated (Wolkin, A. et al) $^3$H-standards. Autoradiograms were analysed using MCID densitometry system. Results were statistically analysed using a one-

way ANOVA followed by a student Newman-Keuls post hoc test.

In situ hybridisation: a 45mer oligonucleotide probe was designed against bases 223-267 of the rat parvalbumin gene (GenBank accession number A819345). *In situ* hybridisation was carried out according to the method of Wisden and Morris (1994).

Table 2: Effect of haloperidol and clozapine on PCP-induced hypofunction

| | LCGU (µmol/100g/min) | | | | | |
|---|---|---|---|---|---|---|
| | vehicle | vehicle Clz | vehicle hal | PCP vehicle | PCP Clz | PCP hal |
| *Prefrontal Cortex* | | | | | | |
| mO layer I | 127±7 | 93±4* | 93±4* | 104±5* | 104±5* | 105±4 |
| m0 layers II & III | 138±6 | 109±4* | 106±4* | 121±6 | 112±6* | 116±4* |
| mO layers V & VI | 135±9 | 106±5* | 102±4* | 113±6 | 115±5* | 119±4 |
| PrL layer I | 139±5 | 119±4* | 114±4* | 109±4* | 118±6* | 115±3* |
| PrL layers II & III | 152±7 | 139±5 | 131±4 | 127±5* | 134±7 | 134±4 |
| PrL layers V & VI | 116±5 | 98±4* | 93±4* | 97±3* | 104±5 | 97±3* |
| *Thalamus* | | | | | | |
| Rt | 112±6 | 95±4* | 86±4* | 79±2* | 81±2* | 80±1* |
| MD | 130±6 | 124±6 | 117±5 | 133±6 | 113±3 | 119±6 |
| *Auditory System* | | | | | | |
| Au layer I | 153±5 | 136±6 | 142±9 | 138±3 | 140±5 | 141±8 |
| Au layers II,III&IV | 183±8 | 169±6 | 167±9 | 174±4 | 166±6 | 174±10 |
| Au layers V & VI | 126±2 | 126±4 | 112±9 | 120±2 | 118±4 | 117±7 |
| AuD layer I | 157±8 | 136±4 | 139±9 | 126±5* | 135±5 | 133±7 |

| | LCGU (µmol/100g/min) | | | | | |
|---|---|---|---|---|---|---|
| | vehicle | vehicle Clz | vehicle hal | PCP vehicle | PCP Clz | PCP hal |
| AuD layers II,III&IV | 170±10 | 154±5 | 153±9 | 141±6* | 152±6 | 156±10 |
| AuD layers V & VI | 122±2 | 115±6 | 108±7 | 105±2* | 107±4 | 106±6* |
| DLL | 112±5 | 99±5 | 92±4 | 89±4* | 108±5 | 107±2 |
| VLL | 120±4 | 109±5 | 106±5 | 98±6* | 118±5 | 110±3 |
| cochlear nucleus | 125±2 | 108±7 | 99±9* | 92±4* | 119±8 | 115±2 |
| *Hippocampus* | | | | | | |
| CA1 molecular layer | 106±3 | 102±5 | 92±5 | 93±2 | 87±2* | 97±4 |
| CA1 stratum | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| radiatum | 82±3 | 80±4 | 66±4* | 67±2* | 66±3* | 74±4 |
| CA1 pyramidal cell layer | 79±3 | 78±4 | 63±4* | 63±2* | 62±2* | 70±4 |
| CA1 stratum oriens | 73±3 | 72±4 | 59±4* | 59±2* | 57±2* | 64±4 |
| CA3 moleular layer | 96±2 | 96±4 | 90±4 | 90±2 | 84±2 | 94±6 |
| CA3 stratum radiatum | 76±2 | 83±5 | 73±1 | 70±3 | 69±2 | 76±5 |
| CA3 pyramidal cell layer | 75±3 | 81±4 | 71±4 | 69±2 | 69±3 | 74±5 |
| CA3 stratum oriens | 69±3 | 74±4 | 64±4 | 60±3 | 62±1 | 69±5 |

Table 2: All data expressed as mean LCGU (µmol/100g/min) ± SEM (n=6). Statistical analysis carried out using individual two-way ANOVA for each discrete brain region followed by Tukey's post hoc test where appropriate, with statistical significant defined as $p < 0.05$. *$p<0.05$ compared to controls. The treatment paradigm was as follows: once daily i.p. injections of PCP (2.58mgkg$^{-1}$) or vehicle (saline) on days 1 to 5 (phase 1), implantation of primed osmotic minipumps on day 8 (vehicle, clozapine 20mgkg$^{-1}$/day, haloperidol 1mgkg$^{-1}$/day), i.p. injections of PCP or vehicle once daily on days 8, 10, 12, 15, 17, 19, 22, 24 and 26 (phase 2) with the animals killed on day 29. Abbreviations are as in Table 1 legend.

## Example 3 - Use of PCP model to discover novel genes potentially important in schizophrenia and its treatment

The PCP model as described herein has been used to identify novel genes for schizophrenia using two different molecular biology approaches.

**1) Atlas Arrays**

Four groups of rats were treated with (a) chronic PCP, (see Example 1), (b) chronic vehicle (control), (c) chronic PCP plus chronic clozapine and (d) chronic PCP plus chronic haloperidol.

Rats were injected with PCP (2.58mg/kg) or vehicle i.p. for 5 days according to the YRING PCP model. On day 7, they were implanted with osmotic minipumps containing either clozapine or haloperidol at concentrations that would administer drugs at 20 or 1mg/kg/day respectively, or vehicle. On the same day, the rats began a course of i.p. injections every 2.5 days with either PCP (2.58mg/kg) or vehicle. This regimen gave the following treatment groups:

| I.P. | Minipump | N° |
|---|---|---|
| Vehicle | Vehicle | 6 |
| PCP | Vehicle | 6 |
| PCP | Clozapine | 6 |
| PCP | Haloperidol | 6 |

21 days after minipump implantation, animals were killed by cervical dislocation and the prefrontal cortex dissected and stored at -70°C. RNA was then prepared according to the protocol below and the corresponding cDNA synthesis and hybridisation procedure were conducted using the rat Atlas Array kit according to the manufacturer's instructions (Clontech). Several genes were affected by

the treatments. Of particular interest was E3C (calcium independent alpha-latrotoxin receptor CIRL) which showed an increase after the PCP treatment regime and which was reversed by the antipsychotic drugs haloperidol and clozapine. A second experiment has been performed using the same treatment regimes with an n=4 per group (each value being pooled prefrontal cortex tissue from 3 rats). Significant increases in CIRL were confirmed after chronic PCP and in addition there were significant increases in expression of UNC5H1 (a netrin receptor) and synapsins (1A and 1B) after chronic PCP as compared to the vehicle treated control group (see Table below)

| Gene | Vehicle control | Chronic PCP | Significance; t test |
|---|---|---|---|
| CIRL-1 | 4542±804 | 9145±669 | P<0.009 |
| UNC5H1 | 1410±480 | 3936±472 | P<0.015 |
| Synapsins 1A&1B | 17365±1144 | 23020±1412 | P<0.025 |

Results are expressed as mean relative optical densities ± SEM. Statistical significance was defined as $P<0.05$. N=3/4 per group.

**Protocol for RNA Preparation for Atlas Arrays**

Frozen tissue already resides in the ribolyser tubes from the dissection procedure

1) Add 1.1ml Qiagen lysis buffer (containing β-

mercaptoethanol, final volume = 3%).

2) Perform 3 x 20sec homogenisations at 6.5g in a ribolyser.

3) Spin 3min in microfuge.

4) Decant to fresh tube and re-spin 3min.

5) Decide at this stage if you want to dilute supernatant with more lysis buffer.

6) Add equal volume of phenol/$CHCl_3$ pH4.7, vortex 30sec and leave on ice for 10min.

7) Re-vortex and spin 4°C 5-10min at 1500g.

8) Decant supernatant and add 100ul $H_2O$. Add an equal volume of phenol/$CHCl_3$ pH4.7, vortex, spin 5-10min at 1500g.

9) Decant supernatant and add 100µl $H_2O$. Add an equal volume of $CHCl_3$, vortex, spin 5-10min at 1500g.

10) Decant supernatant to fresh tube.

11) Re-extract with more lysis buffer and proceed through steps 6-9 and pool fraction with stage 10 (do not add $H_2O$ to supernatants).

12) Measure supernatant volume, add 0.1 vol. 2M NaOAC and 2.5 vol. (total vol.) ethanol, mix and leave at -80°C at least 1hr.

13) Spin 1500g for 15-20min at 4°C.

14) Wash pellet with 70% EtOH.

15) Spin 5min.

16) Decant supernatant, quick spin, remove rest of supernatant with a pipette.

17) Air dry pellet (don't over dry).

18) Re-suspend pellet in 60µl $H_2O$.

19) Measure $OD_{260}$.

20) DNase 1 treat RNA according to the MessageClean (Genhunter) protocol (except perform additional re-extraction with $H_2O$).

21) Re-suspend in as little $H_2O$ as possible (12µl) to keep the RNA concentrated for the Atlas cDNA synthesis step.

22) 20ug of RNA in a final volume of 5µl is used to generate cDNA according to protocols outlined in the Atlas Array manual.

**2) Further verification of the importance of CIRL**

Samples of human schizophrenic brain and age matched control tissue (obtained from Professor G Reynolds, University of Sheffield) were examined by RT-PCR for changes in the expression of CIRL.

In addition, four groups of rats were treated with a) chronic PCP, chronic vehicle (control), c) chronic PCP plus clozapine and d) chronic PCP plus chronic haloperidol as detailed previously in Atlas Array experiment (p.32). RT-PCR for specific isoforms of CIRL was then conducted in the prefrontal cortex.

**Method for brain tissue preparation (rat and human)**

**RT-PCR Protocol**

Isolation of total RNA

1ml of lysis buffer (Qiagen), including 1% β-mercaptoethanol, was added to approximately 50-100mg of brain tissue. Tissue samples were homogenised using a ribolyser (Hybaid) with 3 bursts at 6.5g lasting 20 seconds each. Samples were then spun at room temperature in a microfuge for 3 minutes. The supernatant was removed and re-spun for a further 3 minutes. The supernatant was decanted to a fresh tube to which an equal volume of 70% ethanol was added. The remaining RNA isolation procedure was carried out according to the manufacturer's protocol (Qiagen).

Synthesis of cDNA

Synthesis of cDNA was carried out according to manufacturers protocols (Life Technologies). Briefly 3-5µg of total RNA was reverse transcribed using oligo dT priming. After cDNA synthesis, samples were aliquoted and stored at -70°C. The amount of cDNA in each aliquot would allow a PCR titration at four different cycles with an input RNA template concentration of about 75ng for each PCR reaction.

PCR

Alterations in expression levels were determined by semi-quantitative PCR. Expression levels between different samples were standardised against the amount of β-actin mRNA present in each sample. Briefly, known amounts of template were PCR amplified. Samples were removed over 4 consecutive cycles, however, the first cycle to be removed sometimes varied depending on when logarithmic amplification was detected. Samples were separated on agarose gels and stained with GelStar solution (Flowgen). Results were plotted as the $\log_{10}$ of relative optical density of bands against increasing cycle number. Linear regression analysis was performed. For β-actin titrations, values were obtained from the intersection of the regression lines with the Y-axis. These values were standardised against a single sample. Standardisation coefficients generated at this step were used to standardise the data from target gene expression levels.

**Results**

The levels of CIRL1 mRNA increased in Brodman Area 11 in postmortem schizophrenic brain tissues as compared to controls suggesting that alterations in CIRL may be important in the schizophrenic disease state (see Figure 13).

Analysis of selected specific isoforms of CIRL in rat brain revealed that chronic PCP treatment reduced the

expression of CIRL1, CIRL2 (AB) and CIRL3 (AA) in the prefrontal cortex (see Figures 14, 15 and 16). There was a reversal of the PCP-induced reductions in the level of CIRL1 mRNA by the atypical antipsychotic drug clozapine but not by the typical antipsychotic drug haloperidol. Both drugs reversed the PCP-induced reductions in CIRL2 and CIRL3.

These data support CIRL as a therapeutic target for antipsychotic drug activity.

**3) Differential Display**

Four groups of rats were treated with (a) chronic PCP, (b) chronic vehicle (control), (c) chronic PCP plus chronic clozapine (d) chronic vehicle plus chronic clozapine (as above).

Differential display was performed according to the method of Liang and Pardee (Molecular Biotechnology, 110, 261-267, 1998). Prefrontal cortex tissue was dissected, and total RNA extracted using Qiagen's "RNeasy" kit. An oligo(dT) primer was then used for cDNA synthesis using MMLV reverse transcriptase. The cDNA template obtained was used as a basis for the polymerase chain reaction (PCR) using the Clontech "Delta" differential display kit. Various pairwise combinations of arbitrary primers and "Advantage 2" polymerase were employed according to the Clontech "Delta" differential display kit manual. Differential display products were electrophoresed on 6%

acrylamide gels and exposed to x-ray film. Bands corresponding to cDNA fragments differentially expressed between prefrontal cortex tissue from vehicle-treated animals and PCP-treated animals were excised, and reamplified using the original primers. Differential expression was then confirmed using further prefrontal cortex tissue from these treatment groups. The cDNAs with verified differential expression were sub-cloned and sequenced, and the sequence information obtained subsequently compared with the "DNA Data Bank of Japan" database, for homology with known genes or ESTs.

Three novel sequences were identified (SEQ ID No.s 1, 2, 3 and 4) as being differentially expressed as well as the previously known gene for phosphodiesterase 1α. Further confirmation of changes in expression of the above identified nucleotide sequences was confirmed following chronic PCP treatment of the rat model by semi-quantitative RT-PCR (data not shown).

**4) RT-PCR**

Four groups of rats were treated with (a) chronic PCP, (b) chronic vehicle (control), (c) chronic PCP plus chronic clozapine (as above) or (d) chronic PCP plus chronic haloperidol (as above). The tissue was processed for RNA extraction and RT-PCR as described above, using primers specific for TNFα mRNA.

Acute PCP treatment reduced the levels of TNFα in rat

prefrontal cortex (see Figure 17). This effect was apparent 2hrs and 24hrs following drug treatment.

In groups of rats chronically treated with PCP and antipsychotic drugs (see p.32 for details), PCP in combination with haloperidol was significantly different from the chronic PCP group (see Figure 18).

Further studies revealed a significant increase in TNFα mRNA levels in the Orbital frontal cortex of schizophrenic patents (see Figure 19).

These results implicate TNFα in the development and treatment of schizophrenia.

**Example 4 - Differentially expressed genes in human blood samples using cDNA macroarrays**

**Materials and methods**

Human male blood samples from schizophrenics and healthy volunteers were obtained from Gartnavel Royal Hospital, Glasgow, UK, with consent. The profile of samples are shown in Table 3.

Total RNAs were isolated from human bloods using TRIzol LS Reagent (Gibco/BRL) and treated with DNase I. Four to 8 µg of total RNAs were used as templates for cDNAs. $^{33}P$ radiolabelled cDNAs were hybridised with the Atlas™ Human Cytokine/Receptor Arrays (Clontech). The arrays were washed and then exposed to X-ray films. The

spots on the films were analysed by densitometry. Data were analysed using independent samples t-test. Statistical significance was defined as $p<0.05$.

**Results and discussion**

In this study, only 24 to 93 out of 268 genes could be measured. This could be due to several reasons. Firstly, many cytokines are poorly expressed. Secondly, the efficiency of 1st strand cDNA synthesis could have been low due to usage of total RNA instead of mRNA. Because of the limited amount of samples available, total RNA was utilised. Finally, some membranes had extremely high background which could not be washed out even boiling the membranes.

At first, the expression levels of genes were compared to each of 3 housekeeping genes, ubiquitin, ribosomal protein S9 and phospholipase A2 for the purpose to correcting the amount of input RNA. Slightly different results were obtained when different housekeeping genes were used to standardise signals. So each relative expression level from 3 housekeeping genes was averaged for lowering the deviation. Only epithelial discoidin domain receptor 1, trkE (23 j) showed significant difference between schizophrenics and controls (see Figure 12). This kinase is purported to be a receptor for nerve growth factor and expressed at low levels in most tissues and expression is highest in the brain and lung (Perez et al).

A recent paper showed that trkC mRNA levels in schizophrenics were decreased in the frontal cortex (Schramm et al). TrkC is a high-affinity receptor for neurotrophin-3. Neurotrophins and their receptors have been implicated in the molecular-pathology in schizophrenia (Bayer & Falkai). TrkE might also show the same reduction with trkC.

**Table 3. Profile of human blood samples**

| Schizophrenics | | | | | |
|---|---|---|---|---|---|
| **Code No** | **Smoker** | **Age** | **Weight(kg)** | **Medication** | **Medical History** |
| 01 | No | 30 | 89 | Clz 500 mg/day | 14 yr |
| 02 | No | 40 | 98 | Clz 250 mg/day | 20 yr |
| 25 | Yes | 55 | 70 | Clz 250 mg/day | 20 yr |
| 27 | No | 42 | 103 | Clz 600 mg/day<br>Fpz 75 mg/2weeks | 24 yr |
| 34 | No | 46 | 80 | Clz 100 mg/day<br>Diclofenac Sodium | 23 yr |
| | | 42.6±9.1 | | | |

| Controls | | | | | |
|---|---|---|---|---|---|
| **Code No** | **Smoker** | **Age** | **Weight(kg)** | **Medication** | **Medical History** |
| 04 | Yes | 32 | 76 | - | - |
| 24 | No | 44 | 95 | - | - |
| 28 | No | 27 | 73 | - | - |
| 32 | No | 35 | 84 | - | Sore Throat |
| 35 | No | 37 | 70 | CoProxamol | - |
| | | 35.0±6.3 | | | |

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## CLAIMS

1. Use of one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, for diagnosing schizophrenia.

2. Use of one or more polypeptide sequence(s) derived from one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, for diagnosing schizophrenia.

3. A method of diagnosing schizophrenia, said method comprising using one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, as indicator(s) of schizophrenia.

4. A method of diagnosing schizophrenia, said method comprising using one or more polypeptide sequence(s) derived from one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, as indicator(s) of schizophrenia.

5. Use of one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, in the manufacture of a medicament for the treatment of schizophrenia.

6. Use of one or more polypeptide sequence(s) derived from one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, in the manufacture of a medicament for the treatment of schizophrenia.

7. An isolated polynucleotide sequence having nucleic acid sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4.

8. An isolated nucleic acid having at least 80% identity or homology with a nucleic acid sequence selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4.

9. An isolated nucleic acid according to claim 8, wherein said nucleic acid has at least 90% identity or homology.

10. An isolated nucleic acid according to claim 8, wherein said nucleic acid is at least 15 nucleotides in length.

11. A nucleic acid which can specifically hybridize with a sequence selected from the group consisting of SEQ ID Nos. 1, 2, 3 and 4, or their compliment.

12. A nucleic acid according to claim 11, wherein said nucleic acid has at least 80% sequence identity or homology with a sequence selected from the group consisting of SEQ ID Nos. 1, 2, 3 and 4, or their compliment.

13. A nucleic acid according to claims 11 or 12, wherein said nucleic acid is at least 15 nucleotides in length.

14. Use of a nucleic acid as claimed in claim 13 for diagnosing schizophrenia.

15. A recombinant nucleic acid molecule comprising a polynucleotide fragment as claimed in claims 7 to 13.

16. A recombinant nucleic acid molecule according to claim 15 characterised in that the recombinant nucleic acid molecule comprises regulatory control sequences operably linked to said polynucleotide fragment for controlling expression of said polynucleotide fragment.

17. A recombinant nucleic acid molecule according to claim 16 wherein the recombinant nucleic acid molecule is a plasmid.

18. A recombinant nucleic acid molecule according to claim 16 wherein the recombinant nucleic acid molecule is derived from a viral vector.

19. A prokaryotic or eukaryotic host cell transformed by a polynucleotide fragment or recombinant molecule according to any of claims 7 to 17.

20. Use of one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, in the identification of compounds which modulate the expression of said polynucleotide sequence(s).

21. Use of one or more polypeptide sequence(s) derived from one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, in the identification of compounds which modulate the expression of said polypeptide sequence(s).

22. Use of a polynucleotide or polypeptide sequence according to claims 20 or 21, wherein said identified compound results in overexpression of said polynucleotide or polypeptide sequence.

23. Use of a polynucleotide or polypeptide sequence according to claims 20 or 21, wherein said identified compound results in underexpression of said polynucleotide or polypeptide sequence.

24. An antisense nucleotide fragment complimentary to a polynucleotide fragment or subfragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof.

25. Use of an antisense nucleotide fragment complimentary to a polynucleotide fragment or subfragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, in the manufacture of a medicament for the treatment of schizophrenia.

26. A method for screening a compound which regulates expression of a schizophrenia-related gene(s), said method comprising:

(a) bringing a test compound into contact with transformed cell which enables expression of a gene selected from the group consisting of SEQ ID No. 1, SEQ ID

No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 (PDEI α), SEQ ID No. 7, 9 & 11 (CIRL 1, 2 & 3), SEQ ID No. 13 (trkE), SEQ ID No. 15 (netrin receptor, SEQ ID No. 17 & 19 (aynapsin 1A/AB), and SEQ ID No. 21 (TNFα),

(b) detecting an expression of schizophrenia-relating factor in said cell, and

(c) selecting a compound which promotes or suppresses an induction of the schizophrenia-relating factor in comparison with a control (vehicle).

27. A method for measuring an anti-schizophrenic effects of a compound using the animal model of the present invention, which comprises:

(a) measuring local cerebral glucose utilisation (LCGU), or detecting an expression level of one or more genes represented by the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5 (PDEI α), SEQ ID No. 7, 9 & 11 (CIRL 1, 2 & 3), SEQ ID No. 13 (trkE), SEQ ID No. 15 (netrin receptor, SEQ ID No. 17 & 19 (aynapsin 1A/AB), and SEQ ID No. 21 (TNFα), and

(b) comparing with a control group.

28. A transgenic animal wherein the expression of one or more genes containing nucleotide sequences selected from consisting of SEQ ID Nos. 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 19 and 21 has/have been modulated *in vivo*.

29. A cell line wherein the expression of one or more genes containing nucleotide sequences selected from consisting of SEQ ID. Nos. 1, 2, 3, 4, 5, 7, 9, 11, 13, 15, 17, 19 and 21 has/have been modulated.

30. An antibody immuno-reactive with a polypeptide or fragment thereof derived from a polynucleotide fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 and SEQ ID No. 4.

31. Use of an antibody immuno-reactive with a polypeptide, or fragment thereof, derived from a polynucleotide fragment selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21 for diagnosing schizophrenia.

32. A pharmaceutical composition comprising a polynucleotide fragment, or derivative thereof, according to any of claims 7 to 13 together with a pharmaceutically acceptable carrier.

33. A pharmaceutical composition comprising a polypeptide fragment, or derivative thereof, according to claim 32 together with a pharmaceutically acceptable carrier.

34. Use of one or more polypeptide sequence(s) derived from one or more polynucleotide sequence(s) selected from the group consisting of SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3, SEQ ID No. 4, SEQ ID No. 5, SEQ ID No. 7, 9 and 11, SEQ ID No. 13, SEQ ID No. 15, SEQ ID No. 17 and 19, and SEQ ID No. 21, or fragments thereof, for testing candidate compounds for any effect on said polypeptide(s).

35. A chronic animal model of schizophrenia.

36. A chronic animal model according to claim 35, wherein said animal model has been developed by the addition of PCP to an animal.

37. A method for developing a chronic animal model of schizophrenia said method comprising the steps of:

(a) administering PCP to an animal in order to induce a psychotic state in the animal representative of the onset of schizophrenia in humans; and

(b) further administrating of PCP in order to maintain the PCP-induced psychotic state in the animal,

over a period of time, to mimic a chronic state of schizophrenia in the animal.

38. A method according to claim 37 wherein the dose of PCP used to induce a psychotic state in said animal is in the range of 1 to 5 $mgkg^{-1}$.

39. A method according to claim 38 wherein the dose of PCP used to induce a psychotic state in said animal is in the range of 2 to 4 $mgkg^{-1}$.

40. A method according to claim 39 wherein the dose of PCP used to induce a psychotic state in said animal is about 2.58$mgkg^{-1}$.

41. An animal model produced by the method according to any one of claims 37 to 40.

42. An animal model according to claims 35, 36 or 41 wherein the animal is selected from the group consisting of rat, mouse, guinea pig or rabbit.

43. Use of the animal model as claimed in any of claims 35, 36, 41 or 42 for screening new drugs for the treatment of schizophrenia.

44. Use of the animal model as claimed in any of claims 35, 36, 41 or 42 in the identification of genes associated with the schizophrenic state.

45. A method for screening an atypical antipsychotic drug which comprises using parvalbumin or CIRL1 as an indicator.

FIGURE 1
Gene sequence for YSG 3(SEQ ID NO.1) rat

TCCAGACTCTGAAAGCACACAAGAACGGTCATGGAATCTNAGCAAAGCCTAACCAAGAAA
AGCTCCAGTTCCTCCTGTTCGGCAGGGCGTGGGCATCGGCAGTGCCAGGGAATGCTTGGT
GCATGAACAGGACCCCCAGGTGAGCCATATTTGCAGTAAGAGTCATCAGCATTGCTCCTG
AGAAGCCTCAGGCTCAGAAGAAAGCTTTTGCTAGCAAATTGTTAGGGTCTGGGAAGTAAT
GCTCAGGGCTAGGATAGCATACCCAAGGCCCCGTGCTGCATCCCAACACTG

FIGURE 2
Gene sequence for YSG 4 (SEQ ID No. 2)

AACATTCCAAACAAAAGACACTAATATTTAGGCATGCATGTGATCTTGTTCAACTTCTCT
GTTTTTAGTTATTTGTGTAAACATTATCATTACGAATTGCATTTTTTGAACTTTTCTATT
TTCAGGCAATGAGAACAAATACAGAGGTACAGAACTAAGTATTACACACGCACACACACA
CACACACACACACACACACACACAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA
GAGAGAGAGAGAAGGGAAGGTAAAGGTGGACTTAAAAAACATTGCTCTAAATGGGAAGTC
TCAAGCAAGTCTCTTCACTCAGACCTCGGGGGTCCTTCAT

FIGURE 3
Gene sequence for YSG 6 (SEQ ID No.3) rat

GCAGGATGCAACCGGACATTTCTCCTTTGTAGAGTGAGGATCCCACAGAAGTGTTGTGAT
ACCCGAAGGGCAGCAGCAGGCTGCTGCTGTGTCTGTGATATTAAATCCCATTGTTAATCA
CTAAGGATTAATTGTTAAAGGATAAACACAAGGTGTTTGTTTGGCTCCCAGCAATCTTGA
AATTAAATAAGAAAGGAGGTTTGGGACCAACTCCTGAGTGAGTAGATAGCCCTAGAAGGA
ACTGCTTCACCCAGAACCTGGGTCCCAGGGTTCTAGACCAGGGAGGGGCCAAGCAAGTGA
ATGGTTTTGCCAGGAAGCTGGAACTAAGGAGCTGTTTGGCAGG

Figure 4
Gene sequence for YSG 9 ( SEQ ID No. 4) rat

CACAGCCTCTGTTAAAAGGCATCTGGGTCTTGGTAAATGGCTTTTTATCTGTGTTATTTA
TGTGTCCAACATTTTATGTGTGTGCCGAGTTCAGAGGGTAAGCCCACATGCTACCACAGA
CTCAGCCAGGGAAATCCAGTACAATGGGTCCAAGCACTTAATTCATTAATTTATTTTTGA
GACAGCCACGTGTAGCCCAGGGTGGCTTTAAACTCACAATGTAGCAGAGGCTGGCTTTGA
ACTTTTTATCCTCCTGATTCTAATTCCCATGCTAGAATTAGAGGCTTTTGCCACCA

FIGURE 5a
Gene sequence for YSG1 (SEQ ID No.5)
(phosphodiesterase 1α, rat)

ATGGCAAGACAAGGCTGTCTCGGGTCATTCCAGGTAATATCCTTGTTCAC
TTTTGCCATCAGTGTCAATATCTGCTTAGGATTCACAGCAAGTCGAATTA
AGAGGGCAGAATGGGATGAAGGACCTCCCACAGTGCTGTCTGACTCTCCA

TGGACCAACACCTCTGGATCCTGCAAAGGTAGATGCTTTGAGCTTCAAGA
GGTTGGCCCTCCAGACTGTCGGTGTGACAACCTGTGTAAGAGCTACAGCA
GCTGCTGCCACGATTTCGATGAGCTCTGTTTGAAAACAGTCCGAGGCTGG
GAGTGCACCAAAGACAGAAGTGGGGAAGTACGAAACGAGGAAAATGCCTG
TCACTGCCCAGAAGACTGCTTGTCCAGGGGAGACTGCTGTACCAACTACC
AAGTGGTCTGCAAAGGAGAATCACACTGGGTAGATGATGCTGCGAGAAAT
CAAAGTTCCGAATGCCTGCAGGTTTGTCCGCCTCCGTTAATCATCTTCTC
TGTGGATGGTTTCCGTGCATCATACATGAAGAAAGGCAGCAAGGTTATGC
CCAACATTGAGAAACTGCGGTCCTGTGGCACCCATGTCCCCTACACGAGG
CCTGTGTACCCCACAAAAACCTTCCCTAATCTATATACGCTGGCCACTGG
TTTATATCCGGAATCCCATGGAATTGTCGGTAATTCAATGTATGATCCTG
TCTTTGATGCTTCGTTCCATCTACGAGGGCGAGAGAAGTTTAATCATAGG
TGGTGGGGAGGCCAACCGCTATGGATTACAGCCACCAAGCAAGGGGTGAG
AGCTGGAACATTCTTTTGGTCTGTGAGCATCCCTCATGAACGGAGGATCC
TAACCATTCTTCAGTGGCTTTCTCTGCCAGACAACGAGAGGCCTTCAGTT
TATGCCTTCTACTCAGAGCAGCCTGATTTTTCTGGACACAAGTACGGCCC
TTTTGGCCCTGAGATGACAAATCCTCTGAGGGAGATTGACAAGACCGTGG
GGCAGTTAATGGATGGACTGAAACAACTCAGGCTGCATCGCTGTGTGAAC
GTTATCTTTGTTGGAGACCATGGAATGGAAGATGTGACATGTGACAGAAC
TGAGTTCTTGAGCAACTATCTGACTAATGTGGATGACATTACTTTAGTGC
CTGGAACTCTGGGAAGAATTCGAGCCAAATCTATCAATAATTCTAAATAT
GACCCTAAAACCATTATTGCTAACCTCACGTGCAAAAAACCGGATCAGCA
CTTTAAGCCTTACATGAAACAGCACCTTCCCAAACGGTTGCACTATGCCA
ACAACAGAAGAATTGAAGACATCCATTTATTGGTCGATCGAAGATGGCAT
GTTGCAAGGAAACCTTTGGACGTTTATAAGAAACCATCAGGAAAATGTTT
TTTCCAGGGTGACCACGGCTTTGATAACAAGGTCAATAGCATGCAGACTG
TTTTCGTAGGTTATGGCCCAACTTTTAAGTACAGGACTAAAGTGCCTCCA
TTTGAAAACATTGAACTTTACAATGTTATGTGCGATCTCCTAGGCTTGAA
GCCCGCTCCCAATAATGGAACTCATGGAAGCTTGAATCACCTACTGCGTA
CAAATACCTTTAGGCCAACCATGCCAGACGAAGTCAGCCGACCTAACTAC
CCAGGGATTATGTACCTTCAGTCCGAGTTTGACCTGGGCTGCACCTGTGA
CGATAAGGTAGAGCCAAAGAACAAATTGGAAGAACTCAATAAACGTCTTC
ATACCAAAGGATCAACAGAAGCTGAAACCGGGAAATTCAGAGGCAGCAAA
CATGAAAACAAGAAAAACCTTAATGGAAGTGTTGAACCTAGAAAAGAGAG
ACATCTCCTGTATGGACGGCCTGCAGTGCTCTATCGGACTAGCTATGATA
TCTTATACCATACGGACTTTGAAAGTGGTTATAGTGAAATATTCTTAATG
CCTCTCTGGACATCGTATACCATTTCTAAGCAGGCTGAGGTCTCCAGCAT
CCCAGAACACCTGACCAACTGTGTTCGTCCTGATGTCCGTGTGTCTCCAG
GATTCAGTCAGAACTGTTTAGCTTATAAAAATGATAAACAGATGTCATAT
GGATTCCTTTTTCCTCCCTACCTGAGCTCCTCCCCAGAAGCTAAGTATGA
TGCATTCCTCGTAACCAACATGGTTCCAATGTACCCCGCCTTCAAACGTG
TTTGGGCTTATTTCCAAAGGGTTTTGGTGAAGAAATATGCTTCAGAAAGG
AATGGAGTCAACGTAATAAGTGGACCGATTTTTGACTACAATTACGATGG
CCTACGTGACACTGAAGATGAAATTAAACAGTATGTGGAAGGCAGCTCTA
TACCTGTCCCCACCCACTACTACAGCATCATCACCAGCTGCCTGGACTTC
ACTCAGCCTGCAGACAAGTGTGACGGTCCCCTCTCTGTGTCTTCCTTCAT
CCTTCCTCACCGACCCGACAATGATGAGAGCTGTAATAGCTCCGAGGATG
AGTCGAAGTGGGTAGAGGAACTCATGAAGATGCACACAGCTCGGGTGCGG
GACATTGAGCACCTCACTGGTCTGGATTTCTACCGGAAGACTAGCCGTAG

CTATTCGGAAATTCTGACCCTCAAGACATACCTGCATACATATGAGAGCG
AGATTTAA

FIGURE 5b
Peptide sequence for YSG1 (SEQ ID No.6)
(phosphodiesterase 1α, rat )

MARQGCLGSFQVISLFTFAISVNICLGFTASRIKRAEWDEGPPTVLSDSP
WTNTSGSCKGRCFELQEVGPPDCRCDNLCKSYSSCCHDFDELCLKTVRGW
ECTKDRSGEVRNEENACHCPEDCLSRGDCCTNYQVVCKGESHWVDDAARN
QSSECLQVCPPPLIIFSVDGFRASYMKKGSKVMPNIEKLRSCGTHVPYTR
PVYPTKTFPNLYTLATGLYPESHGIVGNSMYDPVFDASFHLRGREKFNHR
WWGGQPLWITATKQGVRAGTFFWSVSIPHERRILTILQWLSLPDNERPSV
YAFYSEQPDFSGHKYGPFGPEMTNPLREIDKTVGQLMDGLKQLRLHRCVN
VIFVGDHGMEDVTCDRTEFLSNYLTNVDDITLVPGTLGRIRAKSINNSKY
DPKTIIANLTCKKPDQHFKPYMKQHLPKRLHYANNRRIEDIHLLVDRRWH
VARKPLDVYKKPSGKCFFQGDHGFDNKVNSMQTVFVGYGPTFKYRTKVPP
FENIELYNVMCDLLGLKPAPNNGTHGSLNHLLRTNTFRPTMPDEVSRPNY
PGIMYLQSEFDLGCTCDDKVEPKNKLEELNKRLHTKGSTEAETGKFRGSK
HENKKNLNGSVEPRKERHLLYGRPAVLYRTSYDILYHTDFESGYSEIFLM
PLWTSYTISKQAEVSSIPEHLTNCVRPDVRVSPGFSQNCLAYKNDKQMSY
GFLFPPYLSSSPEAKYDAFLVTNMVPMYPAFKRVWAYFQRVLVKKYASER
NGVNVISGPIFDYNYDGLRDTEDEIKQYVEGSSIPVPTHYYSIITSCLDF
TQPADKCDGPLSVSSFILPHRPDNDESCNSSEDESKWVEELMKMHTARVR
DIEHLTGLDFYRKTSRSYSEILTLKTYLHTYESEI

FIGURE 6a
Gene sequences for YSG2 (SEQ ID No.7)( CIRL, rat )
CIRL-1 variant BB ( other variants: AA, AB, BA)

ATGGCCCGCTTGGCTGCAGCACTCTGGAGTCTCTGTGTGACGACTGTCCT
CGTCACCTCTGCTACCCAAGGCCTGAGCCGGGCTGGACTCCCATTTGGAT
TGATGCGCCGGGAGCTAGCATGCGAAGGCTACCCCATTGAGCTGCGGTGC
CCGGGCAGTGACGTCATCATGGTGGAGAATGCAAACTATGGGCGCACAGA
TGACAAGATCTGCGATGCCGACCCTTTTCAGATGGAGAACGTGCAGTGCT
ACCTGCCTGACGCCTTCAAAATCATGTCACAGAGATGTAATAACCGAACC
CAGTGTGTGGTGGTGGCCGGCTCTGACGCCTTTCCTGACCCCTGTCCTGG
AACCTACAAGTACCTGGAGGTGCAGTACGACTGTGTCCCTTACAAAGTGG
AGCAGAAAGTCTTCGTGTGCCCAGGGACACTGCAGAAGGTGCTGGAGCCC
ACCTCCACACATGAATCGGAGCACCAGTCTGGCGCATGGTGCAAGGACCC
ACTGCAGGCAGGTGACCGTATCTACGTTATGCCCTGGATCCCCTACCGCA
CGGACACACTGACCGAGTATGCTTCCTGGGAGGACTATGTGGCTGCACGC
CACACCACCACGTACAGACTGCCCAACCGTGTAGATGGCACTGGCTTTGT
GGTATATGATGGTGCCGTCTTCTATAACAAGGAACGTACTCGCAACATTG
TCAAATATGACCTGCGGACCCGCATCAAGAGCGGAGAAACAGTCATAAAC
ACAGCCAACTACCACGACACCTCACCTTATCGCTGGGGAGGCAAAACCGA
CATTGACCTGGCAGTGGATGAGAACGGGCTGTGGGTCATCTATGCCACCG

AGGGGAACAACGGGCGTCTGGTGGTGAGCCAGCTCAACCCCTACACACTG
CGTTTCGAGGGCACCTGGGAAACAGGCTATGACAAGCGCTCAGCCTCCAA
TGCCTTCATGGTGTGTGGTGTCCTCTATGTGCTGCGCTCTGTTTATGTGG
ATGACGACAGTGAGGCAGCAGGCAACCGCGTGGACTATGCCTTTAACACC
AATGCAAACCGAGAGGAGCCCGTCAGTCTCGCCTTCCCCAACCCCTACCA
GTTTGTATCTTCTGTTGACTACAATCCCCGGGACAACCAGCTGTATGTGT
GGAACAACTATTTCGTGGTGCGCTACAGCCTGGAGTTTGGACCCCCAGAT
CCCAGTGCTGGCCCAGCCACTTCCCCACCTCTCAGTACCACCACCACAGC
TCGGCCTACGCCCCTCACCAGCACAGCCTCACCTGCAGCCACCACTCCAC
TCCGCCGGGCGCCCCTCACCACGCACCCAGTAGGTGCCATCAACCAGCTG
GGACCTGACCTGCCTCCAGCCACAGCCCCAGCACCCAGTACCCGGCGGCC
TCCAGCCCCCAATCTGCATGTGTCCCCTGAGCTCTTCTGTGAACCCCGAG
AGGTCCGGCGGGTCCAGTGGCCAGCTACCCAGCAGGGTATGCTGGTAGAG
AGACCTTGCCCCAAGGGAACTCGAGGAATTGCCTCGTTCCAGTGCCTCCC
AGCTCTGGGGCTCTGGAATCCTCGGGGCCCTGACCTCAGCAACTGCACTT
CCCCCTGGGTCAACCAAGTCGCCCAGAAGATCAAGAGTGGAGAGAATGCA
GCCAACATTGCTAGTGAGCTGGCCCGCCACACGCGGGGCTCCATCTATGC
TGGGGACGTGTCCTCATCGGTGAAGCTGATGGAGCAACTGCTAGATATCC
TGGATGCCCAGCTCCAGGCCCTACGGCCCATTGAACGAGAGTCAGCTGGC
AAGAACTACAATAAGATGCACAAGCGAGAGAGAACCTGCAAGGACTATAT
CAAGGCTGTGGTGGAGACAGTGGACAACCTGCTTCGGCCAGAGGCACTTG
AGTCATGGAAAGACATGAATGCCACCGAACAGGTCCATACGGCCACCATG
CTCCTAGATGTCTTAGAGGAGGGTGCCTTCCTGCTGGCCGACAATGTCAG
AGAACCTGCTCGCTTCTTGGCTGCCAAGCAGAATGTGGTCCTGGAGGTCA
CTGTCCTGAGCACAGAGGGTCAAGTGCAGGAGTTGGTGTTCCCCCAGGAG
TATGCCAGTGAGAGCTCCATTCAGCTGTCCGCCAACACCATCAAGCAGAA
CAGCCGCAATGGTGTGGTGAAGGTTGTCTTCATTCTCTACAACAACCTGG
GCCTCTTCTTGTCCACGGAGAATGCCACAGTGAAGCTGGCAGGTGAGGCA
GGGACCGGTGGCCCTGGAGGTGCCTCCCTGGTGGTTAACTCACAGGTCAT
CGCAGCATCCATCAATAAGGAGTCCAGCCGTGTCTTCCTCATGGACCCTG
TCATCTTTACTGTGGCCCACTTGGAGGCCAAGAACCACTTCAATGCAAAC
TGCTCCTTCTGGAACTACTCAGAGCGCTCCATGCTGGGCTACTGGTCAAC
CCAGGGCTGCCGACTGGTGGAGTCCAATAAGACCCATACCACATGTGCCT
GCAGCCACCTCACCAACTTCGCAGTGCTCATGGCTCACCGAGAGATCTAC
CAAGGCCGTATTAATGAGCTGTTGCTGTCAGTCATCACCTGGGTTGGCAT
TGTCATCTCCCTGGTCTGTCTGGCTATCTGCATCTCCACCTTCTGCTTCC
TGCGGGGCCTGCAGACCGACCGCAACACCATCCACAAGAACCTGTGCATC
AACCTCTTCCTTGCAGAGCTGCTCTTCCTGGTTGGAATAGACAAAACTCA
GTATGAGGTCGCCTGCCCTATCTTTGCGGGCCTGCTGCACTACTTCTTCC
TGGCCGCCTTCTCCTGGCTGTGCCTAGAGGGCGTGCACCTCTACCTCCTG
CTGGTCGAGGTGTTCGAGAGCGAATATTCACGCACCAAGTACTATTACCT
GGGCGGCTACTGCTTCCCAGCCCTGGTGGTAGGCATCGCAGCCGCCATTG
ACTACCGAAGCTACGGCACTGAGAAGGCCTGCTGGCTGAGGGTGGATAAC
TATTTCATCTGGAGCTTCATTGGGCCCGTCTCCTTTGTTATTGTGGTGAA
CCTGGTGTTCCTCATGGTGACCCTGCACAAGATGATCCGAAGCTCATCCG
TGCTCAAGCCTGACTCCAGCCGCCTTGACAACATCAAGTCCTGGGCGCTG
GGTGCCATTGCACTGCTCTTCCTGCTGGGCCTCACCTGGGCTTTCGGCCT
CCTCTTCATCAACAAGGAGTCAGTAGTAATGGCTTACCTCTTCACAACCT
TCAACGCCTTCCAGGGGGTCTTCATCTTTGTCTTTCACTGCGCCTTACAG

AAAAAGGTGCACAAGGAGTACAGCAAGTGCCTGCGTCACTCCTACTGCTG
CATTCGCTCCCCACCTGGGGGGGCTCACGGCTCCCTTAAGACCTCAGCCA
TGCGAAGTAACACCCGCTACTACACAGGGACCCAGGTACCCGGGCAGGGA
AGGCATATCCACCAGGTCTCTCTGGGGCCGAGAGGCAGGAGTGCTCTGCC
AGAGTCTCAGAAAGATCCTGGAGGGCAGAGTGGTCCTGGAGACCCCCTCA
CGTTTGGGCTGTGTCCCAGCCGAATCCGGAGGATGTGGAATGACACCGTG
AGGAAGCAGACAGAGTCGTCCTTTATGGCAGGGGACATCAACAGCACCCC
CACCCTGAACCGAGGTACCATGGGGAACCACCTACTGACCAACCCTGTGC
TACAGCCCCGTGGGGGCACTAGCCCATACAATACACTCATTGCAGAGTCT
GTGGGCTTCAATCCCTCCTCGCCCCCAGTCTTCAACTCCCCAGGAAGCTA
CAGGGAACCTAAGCACCCCTTGGGCGGCCGGGAAGCCTGTGGCATGGACA
CACTGCCCCTTAATGGCAACTTCAACAACAGCTACTCCTTGCGAAGTGGT
GATTTCCCTCCGGGGGATGGGGGTCCTGAGCCACCCCGAGGCCGAAACCT
AGCGGATGCTGCGGCCTTTGAGAAGATGATCATCTCAGAGCTGGTGCACA
ACAACCTTCGGGGGGCCAGTGGGGGCGCCAAAGGTCCTCCACCAGAGCCT
CCTGTGCCACCCGTGCCAGGAGTCAGTGAGGACGAGGCTGGTGGGCCTGG
GGGTGCTGACCGGGCTGAGATTGAACTTCTCTACAAGGCCCTGGAGGAGC
CACTGCTGCTGCCCCGGGCCCAGTCGGTGCTGTACCAGAGTGATCTGGAT
GAGTCGGAGAGCTGTACGGCAGAGGATGGGGCCACCAGCCGGCCCCTCTC
CTCCCCTCCCGGCCGGGACTCCCTCTATGCCAGCGGGGCCAACCTGCGGG
ACTCGCCCTCCTACCCGGACAGCAGCCCCGAAGGGCCTAATGAGGCCCTG
CCCCCTCCCCCACCTGCTCCCCCTGGGCCCCCAGAAATCTACTACACCTC
TCGCCCGCCGGCCCTGGTGGCTCGGAATCCCCTACAGGGCTACTACCAGG
TGCGGCGGCCCAGCCATGAGGGCTACCTGGCAGCCCCCAGCCTTGAGGGG
CCAGGGCCCGATGGGGATGGGCAAATGCAGTTGGTCACTAGTCTCTGA

FIGURE 6b

Peptide sequences for YSG2 (SEQ ID No.8)( CIRL, rat )
CIRL-1 variant BB

MARLAAALWSLCVTTVLVTSATQGLSRAGLPFGLMRRELACEGYPIELRC
PGSDVIMVENANYGRTDDKICDADPFQMENVQCYLPDAFKIMSQRCNNRT
QCVVVAGSDAFPDPCPGTYKYLEVQYDCVPYKVEQKVFVCPGTLQKVLEP
TSTHESEHQSGAWCKDPLQAGDRIYVMPWIPYRTDTLTEYASWEDYVAAR
HTTTYRLPNRVDGTGFVVYDGAVFYNKERTRNIVKYDLRTRIKSGETVIN
TANYHDTSPYRWGGKTDIDLAVDENGLWVIYATEGNNGRLVVSQLNPYTL
RFEGTWETGYDKRSASNAFMVCGVLYVLRSVYVDDDSEAAGNRVDYAFNT
NANREEPVSLAFPNPYQFVSSVDYNPRDNQLYVWNNYFVVRYSLEFGPPD
PSAGPATSPPLSTTTTARPTPLTSTASPAATTPLRRAPLTTHPVGAINQL
GPDLPPATAPAPSTRRPPAPNLHVSPELFCEPREVRRVQWPATQQGMLVE
RPCPKGTRGIASFQCLPALGLWNPRGPDLSNCTSPWVNQVAQKIKSGENA
ANIASELARHTRGSIYAGDVSSSVKLMEQLLDILDAQLQALRPIERESAG
KNYNKMHKRERTCKDYIKAVVETVDNLLRPEALESWKDMNATEQVHTATM
LLDVLEEGAFLLADNVREPARFLAAKQNVVLEVTVLSTEGQVQELVFPQE
YASESSIQLSANTIKQNSRNGVVKVVFILYNNLGLFLSTENATVKLAGEA
GTGGPGGASLVVNSQVIAASINKESSRVFLMDPVIFTVAHLEAKNHFNAN
CSFWNYSERSMLGYWSTQGCRLVESNKTHTTCACSHLTNFAVLMAHREIY
QGRINELLLSVITWVGIVISLVCLAICISTFCFLRGLQTDRNTIHKNLCI

NLFLAELLFLVGIDKTQYEVACPIFAGLLHYFFLAAFSWLCLEGVHLYLL
LVEVFESEYSRTKYYYLGGYCFPALVVGIAAAIDYRSYGTEKACWLRVDN
YFIWSFIGPVSFVIVVNLVFLMVTLHKMIRSSSVLKPDSSRLDNIKSWAL
GAIALLFLLGLTWAFGLLFINKESVVMAYLFTTFNAFQGVFIFVFHCALQ
KKVHKEYSKCLRHSYCCIRSPPGGAHGSLKTSAMRSNTRYYTGTQVPGQG
RHIHQVSLGPRGRSALPESQKDPGGQSGPGDPLTFGLCPSRIRRMWNDTV
RKQTESSFMAGDINSTPTLNRGTMGNHLLTNPVLQPRGGTSPYNTLIAES
VGFNPSSPPVFNSPGSYREPKHPLGGREACGMDTLPLNGNFNNSYSLRSG
DFPPGDGGPEPPRGRNLADAAAFEKMIISELVHNNLRGASGGAKGPPPEP
PVPPVPGVSEDEAGGPGGADRAEIELLYKALEEPLLLPRAQSVLYQSDLD
ESESCTAEDGATSRPLSSPPGRDSLYASGANLRDSPSYPDSSPEGPNEAL
PPPPPAPPGPPEIYYTSRPPALVARNPLQGYYQVRRPSHEGYLAAPSLEG
PGPDGDGQMQLVTSL

FIGURE 6c
Gene sequences for YSG2 (SEQ ID No.9)( CIRL, rat )
CIRL-2 variant BC (other variants: AA, AB, AC, BA, BB)

ATGGTGTCTTCTGGTTGCAGAATGCGAAGTCTCTGGTTTATCATGATAAT
CAGTTTCTCACCGAATACCGAAGGTTTCAGCAGAGCAGCCTTGCCATTCG
GGTTAGTTAGACGAGAGCTGTCCTGTGAAGGTTATTCTATAGACCTGCGA
TGTCCGGGCAGTGACGTCATCATGATCGAGAGCGCAAACTACGGTCGGAC
GGACGACAAGATCTGCGACGCAGACCCCTTTCAGATGGAGAACACAGACT
GCTACCTCCCTGATGCCTTCAAAATCATGACTCAAAGGTGCAACAACCGA
ACACAGTGTGTAGTAGTTACCGGGTCAGATGTATTTCCTGATCCATGTCC
CGGAACTTACAAATACCTTGAAGTTCAATATGAATGTGTCCCTTACATGG
AGCAAAAAGTTTTTGTGTGTCCTGGAACCTTGAAAGCAATTGTGGACTCT
CCAAGTATCTATGAAGCTGAGCAAAAGGCAGGTGCTTGGTGCAAGGACCC
CCTTCAGGCTGCAGATAAAATTTATTTTATGCCCTGGACTCCCTACCGCA
CCGATACCTTAATAGAATATGCTTCTTTAGAAGATTTTCAAAACAGCCGC
CAGACAACAACATACAAACTTCCAAACCGAGTGGACGGTACTGGATTTGT
GGTGTATGACGGGGCAGTCTTCTTCAACAAAGAAAGAACGAGAAACATTG
TTAAATTTGACTTGAGGACTAGAATCAAGAGTGGGGAGGCCATAATCAAC
TACGCCAACTACCATGACACCTCACCCTACAGATGGGGGGGGAAGACTGA
CATTGACCTGGCAGTGGATGAAAATGGCTTGTGGGTCATCTACGCCACCG
AGCAGAACAACGGAATGATCGTGATTAGCCAGCTCAATCCGTACACTCTC
CGATTCGAAGCAACCTGGGAGACGACGTATGACAAGCGTGCGGCGTCCAA
TGCTTTCATGATATGCGGGGTCCTCTACGTGGTCAGGTCAGTGTACCAAG
ACAATGAAAGCGAAGCTGGCAAGAACGTCATCGACTACATTTACAACACA
AGGTTGAGCCGGGGAGAGCACGTGGACGTTCCCTTCCCCAACCAGTACCA
GTACATCGCTGCAGTGGATTACAACCCAAGAGACAACCAACTCTACGTAT
GGAACAATAACTTTATCTTACGGTATTCTCTGGAGTTTGGTCCACCCGAC
CCTGCCCAAGTGCCTACCACAGCTGTGACAATAACTTCTTCAGCTGAGCT
GTTCAAAACCACAGTGTCAACCACAAGCAGTACTTCACAGAGAGGCCCCG
TGAGCAGCACAGTCGCTGGTCCTCAGGAAGGAAGCCGAGGGACAAAGCCA
CCTCCAGCAGTCTCTACAACCAAAATTCCTCCTGTAACAAATATTTTTCC
CCTGCCAGAGAGATTCTGCGAAGCGTTAGAAATGAAGGGGATAAAGTGGC
CTCAGACACAAAGGGGGATGATGGTTGAGCGACCGTGTCCCAAGGGAACA

AGAGGAACGGCCTCGTATCTCTGCATGGCTTCCACAGGAACCTGGAACCC
GAAGGGCCCGGATCTTAGCAACTGCACCTCTCACTGGGTGAATCAGCTGG
CCCAGAAGATCAGAAGTGGAGAGAATGCTGCAAGTCTGGCCAACGAACTG
GCTAAGCACACCAAGGGGACGGTGTTCGCTGGGGATGTGAGCTCCTCTGT
GAGACTGATGGAACAGTTGGTGGACATCCTGGATGCCCAGCTGCAGGAGC
TGAAACCGAGCGAGAAGGACTCGGCCGGGAGGAGTTATAACAAGCTCCAA
AAACGAGAGAAGACATGCAGGGCTTACCTTAAGGCCATTGTGGACACAGT
AGATAACCTTCTGAGAGCCGAGACTTTGGACTGCTGGAAACACATGAATT
CCTCAGAGCAGGCGCACACAGCCACCATGCTGTTGGACACCTTGGAAGAA
GGAGCATTTGTCCTGGCAGACAACCTTTTGGAACCAACCCGGGTCTCAAT
GCCAACGGATAATATTGTTCTAGAAGTCGCTGTCCTCAGCACGGAAGGAC
AGGTCCAAGACTTCACCTTCCATCTCGGCTTCAAGGGGGCCTTCAGCTCC
ATCCAGCTCTCAGCCAACACCGTCAAGCAAAACAGCAGAAACGGGCTGGC
AAAGGTGGTATTCATCATTTACCGGAGTCTGGGACCATTCCTGAGCACCG
AAAATGCGACCGTCAAACTGGGCGCAGACCTCCTGGGTCGGAACAGCACC
ATCGCAGTGAACTCGCACGTCCTTTCAGTCTCCATCAATAAGGAGTCCAG
CCGTGTGTACTTGACAGACCCGGTGCTTTTTTCAATGCCACACATTGATT
CTGACAATTATTTCAACGCAAACTGCTCCTTCTGGAACTACTCAGAGAGA
ACCATGATGGGATATTGGTCTACCCAGGGCTGCAAGCTGGTTGACACTAA
TAAAACTCGCACGACGTGTGCATGCAGCCACCTAACCAATTTTGCTATTC
TCATGGCCCACAGGGAAATTGTGTACAAAGATGGCGTCCACAAATTGCTG
CTGACAGTCATCACCTGGGTGGGCATCGTTGTCTCCCTCGTCTGCCTGGC
TATCTGCATCTTCACCTTCTGCTTCTTCCGAGGCCTGCAAAGCGACCGCA
ACACGATCCACAAGAACCTGTGTATCAACCTCTTCATCGCTGAGTTTATT
TTCCTAATAGGCATTGATAAAACACAGTACACGATTGCGTGCCCCGTGTT
TGCAGGACTCCTGCACTTTTTCTTCCTGGCTGCTTTTTCCTGGATGTGCC
TAGAAGGTGTGCAGCTCTACCTCATGTTGGTTGAAGTTTTCGAGAGTGAA
TACTCAAGGAAGAAGTATTACTATGTCGCCGGGTACCTCTTCCCTGCCAC
AGTGGTCGGTGTTTCAGCTGCTATCGACTACAAGAGTTACGGGACACTAG
AGGCTTGCTGGCTTCACGTTGATAACTATTTCATATGGAGTTTCATTGGG
CCTGTTACTTTCATCATTCTGCTAAATATTATTTTCCTGGTGATCACGCT
GTGCAAAATGGTGAAACATTCAAACACTTTGAAACCAGATTCTAGCAGGT
TGGAAAACATTAATAATTACCGTGTTTGTGATGGATACTATAATACGGAC
TTACCTGGGTCTTGGGTGCTCGGTGCGTTCGCCCTGCTGTGTCTCCTGGG
CCTAACCTGGTCCTTTGGGTTGCTTTTTGTTAACGAGGAGACCGTTGTCA
TGGCTTATCTCTTCACCGCCTTTAATGCTTTCCAGGGACTGTTTATTTTC
ATCTTCCACTGTGCTCTTCAAAAGAAAGTACGGAAAGAGTATGCCAAGTG
CTTCAGACACTGGTACTGCTGTGGTGGCCTCCCGACCGAGAGCCCGCACA
GCTCTGTAAAGGCGTCCACCTCCCGCACCAGTGCTCGTTACTCCTCTGGT
ACACAGAGCCGTATAAGAAGGATGTGGAATGACACCGTGAGGAAGCAGTC
TGAATCGTCTTTTATCTCAGGTGACATCAATAGCACTTCTACCCTTAATC
AAGGAATGACTGGCAATTACCTACTAACAAACCCTCTTCTTCGACCCCAC
GGCACTAACAACCCCTATAACACATTGCTCGCTGAAACAGTTGTATGTAA
TGCCCCTTCAGCGCCCGTGTTTAACTCACCAGGACATTCACTGAACAATA
CCCGGGACACCAGCGCCATGGATACTCTACCGCTAAATGGTAACTTCAAC
AACAGCTACTCCCTGCGCAAGGCCGACTACCACGACGGCGTGCAGGTTGT
GGACTGTGGACTAAGTCTGAACGACACCGCGTTTGAGAAAATGATCATTT
CAGAGTTAGTGCACAACAACCTCCGGGGTAGCAACAAAACCCACAACTTG
GAGCTCAAGCTCCCAGTTAAACCCGTGATTGGCGGCAGCAGCAGCGAAGA

TGACGCGATCGTGGCCGACGCCTCATCTTTGATGCACGGTGATAACCCAG
GGCTGGAATTCCGCCACAAAGAGCTGGAGGCACCGCTCATCCCTCAGCGG
ACTCACTCGCTTCTGTACCAACCCCAGAAAAAAGTGAAACCCGAGGCAAC
CGACAGCTACGTCTCCCAGCTGACGGCCGAGGCCGACGAGCACCTCCAGT
CCCCCAACAGAGACTCTCTGTACACGAGCATGCCCAACCTAAGAGACTCT
CCCTACCCGGAGAGCAGCCCGGACATGGCAGAGGACCTGTCTCCCTCCAG
GAGGAGCGAGAACGAGGACATTTACTACAAAAGTATGCCCAATCTTGGGG
CTGGCCGCCAGCTCCAGATGTGCTACCAGATCAGCAGAGGCAATAGCGAT
GGCTACATCATCCCCATTAACAAAGAAGGGTGCATCCCAGAGGGGGACGT
CAGGGAAGGACAGATGCAGCTGGTAACAAGTCTTTAA

FIGURE 6d

Peptide sequences for YSG2 (SEQ ID No.10) ( CIRL, rat )
CIRL-2 variant BC

MVSSGCRMRSLWFIMIISFSPNTEGFSRAALPFGLVRRELSCEGYSIDLR
CPGSDVIMIESANYGRTDDKICDADPFQMENTDCYLPDAFKIMTQRCNNR
TQCVVVTGSDVFPDPCPGTYKYLEVQYECVPYMEQKVFVCPGTLKAIVDS
PSIYEAEQKAGAWCKDPLQAADKIYFMPWTPYRTDTLIEYASLEDFQNSR
QTTTYKLPNRVDGTGFVVYDGAVFFNKERTRNIVKFDLRTRIKSGEAIIN
YANYHDTSPYRWGGKTDIDLAVDENGLWVIYATEQNNGMIVISQLNPYTL
RFEATWETTYDKRAASNAFMICGVLYVVRSVYQDNESEAGKNVIDYIYNT
RLSRGEHVDVPFPNQYQYIAAVDYNPRDNQLYVWNNNFILRYSLEFGPPD
PAQVPTTAVTITSSAELFKTTVSTTSSTSQRGPVSSTVAGPQEGSRGTKP
PPAVSTTKIPPVTNIFPLPERFCEALEMKGIKWPQTQRGMMVERPCPKGT
RGTASYLCMASTGTWNPKGPDLSNCTSHWVNQLAQKIRSGENAASLANEL
AKHTKGTVFAGDVSSSVRLMEQLVDILDAQLQELKPSEKDSAGRSYNKLQ
KREKTCRAYLKAIVDTVDNLLRAETLDCWKHMNSSEQAHTATMLLDTLEE
GAFVLADNLLEPTRVSMPTDNIVLEVAVLSTEGQVQDFTFHLGFKGAFSS
IQLSANTVKQNSRNGLAKVVFIIYRSLGPFLSTENATVKLGADLLGRNST
IAVNSHVLSVSINKESSRVYLTDPVLFSMPHIDSDNYFNANCSFWNYSER
TMMGYWSTQGCKLVDTNKTRTTCACSHLTNFAILMAHREIVYKDGVHKLL
LTVITWVGIVVSLVCLAICIFTFCFFRGLQSDRNTIHKNLCINLFIAEFI
FLIGIDKTQYTIACPVFAGLLHFFFLAAFSWMCLEGVQLYLMLVEVFESE
YSRKKYYYVAGYLFPATVVGVSAAIDYKSYGTLEACWLHVDNYFIWSFIG
PVTFIILLNIIFLVITLCKMVKHSNTLKPDSSRLENINNYRVCDGYYNTD
LPGSWVLGAFALLCLLGLTWSFGLLFVNEETVVMAYLFTAFNAFQGLFIF
IFHCALQKKVRKEYAKCFRHWYCCGGLPTESPHSSVKASTSRTSARYSSG
TQSRIRRMWNDTVRKQSESSFISGDINSTSTLNQGMTGNYLLTNPLLRPH
GTNNPYNTLLAETVVCNAPSAPVFNSPGHSLNNTRDTSAMDTLPLNGNFN
NSYSLRKADYHDGVQVVDCGLSLNDTAFEKMIISELVHNNLRGSNKTHNL
ELKLPVKPVIGGSSSEDDAIVADASSLMHGDNPGLEFRHKELEAPLIPQR
THSLLYQPQKKVKPEATDSYVSQLTAEADEHLQSPNRDSLYTSMPNLRDS
PYPESSPDMAEDLSPSRRSENEDIYYKSMPNLGAGRQLQMCYQISRGNSD
GYIIPINKEGCIPEGDVREGQMQLVTSL

FIGURE 6e

Gene sequences for YSG2 (SEQ ID No.11)( CIRL, rat )
CIRL-3 variant BA ( other variants: AA, AB, AC, BB, BC)

ATGTGTCCACCTCAGCTGTTCATCCTCATGATGCTTTTAGCACCTGTAGT
TCATGGTGGCAAGCACAATGAGAGACATCCAGCCCTCGCTGCTCCACTGC
GACATGCTGAGCACAGCCCAGGAGGCCCTCTCCCTCCCAGACATCTTCTT
CAGCAGCCAGCTGCAGAGCGCTCTACAGCTCATCGAGGACAAGGGCCACG
TGGAACTGCCAGAGGAGTTCGCGGACCCGGTGCCCCAGGAGCACAGATTG
CAGCCCAAGCTTTCAGCCGTGCCCCAATTCCCATGGCAGTGGTCCGCAGA
GAGCTCTCCTGTGAGAGCTACCCCATTGAGCTACGCTGTCCAGGCACAGA
CGTCATCATGATCGAAAGCGCCAACTACGGGAGGACAGATGACAAGATCT
GTGACTCGGACCCTGCTCAGATGGAGAATATTCGGTGTTATCTGCCAGAT
GCCTATAAGATTATGTCTCAAAGATGCAATAACAGAACCCAGTGTGCAGT
GGTGGCAGGTCCTGATGTATTTCCAGACCCATGTCCGGGAACATATAAAT
ACCTTGAAGTGCAGTATGAATGTGTCCCTTACAAAGTGGAACAAAAAGTT
TTTCTTTGTCCCGGACTGCTCAAAGGAGTGTACCAGAGCGAACACTTGTT
TGAATCTGACCACCAATCTGGGGCATGGTGCAAAGACCCTCTACAGGCTT
CTGACAAGATTTACTATATGCCCTGGACTCCCTACAGAACCGATACCCTG
ACAGAGTATTCATCCAAAGATGACTTCATTGCTGGAAGGCCGACAACTAC
ATACAAGCTCCCTCACAGAGTGGATGGTACTGGATTTGTAGTATATGATG
GTGCCCTGTTCTTCAACAAGGAGCGTACAAGGAACATAGTAAAGTTTGAT
TTGAGGACTAGGATAAAGAGTGGAGAGGCAATCATAGCAAATGCTAACTA
CCATGATACCTCCCCATACCGATGGGGTGGCAAGTCCGACATAGACTTGG
CAGTGGATGAAAACGGATTATGGGTAATCTATGCAACAGAACAGAACAAT
GGCAAAATTGTTATTAGCCAGTTGAACCCTTACACCCTACGGATTGAGGG
GACATGGGACACTGCCTATGATAAAAGGTCTGCTTCCAATGCATTTATGA
TTTGTGGGATTCTGTATGTGGTCAAGTCTGTATATGAGGATGACGACAAT
GAGGCCACCGGTAATAAGATTGACTACATTTACAATACTGACCAAAGCAA
GGATAGCCTGGTGGATGTACCCTTTCCCAACTCATACCAGTACATAGCAG
CCGTGGACTACAATCCCAGGGACAATCTGCTGTACGTGTGGAACAACTAC
CATGTTGTCAAATACTCCTTGGACTTCGGGCCTCTGGATAGCAGATCAGG
GCCAGTGCATCATGGACAAGTTTCCTACATCTCTCCACCGATTCACCTTG
ACTCTGACCTGGAAAGGCCCCCTGTCAGAGGGATTTCTACCACAGGACCC
CTGGGTATGGGAAGCACGACCACCAGCACCACCCTCCGGACTACCACCTG
GAACCTAGGGAGGAGTACAACGCCATCCTTGCCTGGCAGAAGAAACCGCA
GTACCAGTACGCCGTCCCCAGCGATTGAGGTGCTGGATGTTACCACACAC
CTGCCATCTGCAGCCTCCCAAATCCCAGCGATGGAAGAGAGCTGCGAGGC
TGTGGAAGCCCGAGAGATCATGTGGTTTAAGACCCGACAGGGGCAAGTAG
CAAAGCAGTCATGCCCAGCAGGAACCATAGGTGTATCAACTTACCTGTGT
CTTGCTCCTGATGGAATATGGGATCCCCAAGGACCAGATCTCAGCAACTG
CTCTTCTCCTTGGGTCAATCACATAACACAGAAGCTGAAATCTGGAGAAA
CAGCTGCCAATATTGCCAGAGAGCTAGCAGAACAGACCAGAAATCATTTG
AACGCCGGGGATATCACCTACTCAGTTCGTGCCATGGACCAACTGGTTGG
CCTCCTGGACGTACAGCTCAGGAATTTGACACCAGGGGGGAAGGACAGTG
CTGCCCGAAGCTTGAACAAGCTTCAGAAAAGAGAGCGCTCTTGCAGAGCC
TATGTCCAGGCGATGGTGGAGACAGTTAACAATCTCCTTCAGCCACAAGC
TCTGAATGCGTGGAGGGACCTGACGACAAGTGATCAACTACGCGCAGCCA
CCATGTTGCTCGACACTGTGGAGGAGAGTGCTTTCGTGTTAGCCGATAAC

CTTTTGAAGACCGACATTGTCAGGGAGAATACAGACAATATTCAGTTGGA
GGTTGCAAGGCTGAGCACGGAAGGAAACCTAGAAGATCTAAAATTTCCAG
AAAACACGGGCCACGGAAGCACTATACAGCTTTCCGCAAACACGTTAAAG
CAAAATGGCCGGAATGGAGAGATTAGAGTGGCCTTTGTCCTGTATAACAA
CCTGGGTCCTTATTTATCTACGGAGAATGCCAGTATGAAGTTGGGCACAG
AAGCTATGTCCACAAATCACTCAGTTATCGTCAATTCCCCTGTTATTACA
GCAGCAATAAATAAGGAATTCAGTAATAAAGTGTATTTGGCTGATCCTGT
GGTATTTACTGTTAAACATATCAAGCAGTCAGAGGAAAATTTCAACCCTA
ACTGTTCATTTTGGAGCTATTCCAAGCGCACAATGACAGGTTATTGGTCA
ACACAAGGCTGTCGACTCCTGACAACGAACAAGACACACACTACGTGCTC
CTGTAACCACCTCACCAACTTCGCAGTATTAATGGCACATGTGGAAGTTA
AGCACAGCGATGCCGTCCACGATCTTCTTCTGGATGTGATCACGTGGGTC
GGAATCCTGTTGTCTCTTGTTTGTCTCCTGATCTGCATCTTCACATTCTG
CTTCTTCCGTGGGCTCCAGAGCGACCGTAACACCATTCACAAGAACCTGT
GCATCAGCCTGTTTGTGGCAGAACTGCTCTTCCTGATTGGGATCAACAGA
ACCGACCAACCGATTGCCTGTGCAGTGTTTGCGGCTCTTTTGCATTTCTT
CTTCTTGGCGGCCTTCACCTGGATGTTTCTAGAAGGGGTACAGCTGTATA
TCATGCTGGTGGAGGTCTTTGAGAGTGAGCATTCCCGTAGGAAGTACTTC
TATCTGGTTGGCTACGGGATGCCCGCACTCATCGTGGCCGTTTCTGCTGC
AGTCGACTACAGGAGCTATGGAACAGACAAAGTATGTTGGCTTCGCCTTG
ACACCTACTTCATTTGGAGTTTTATAGGACCAGCGACCTTGATAATTATG
CTGAATGTAATCTTCCTCGGGATTGCTTTATACAAAATGTTTCACCATAC
TGCCATACTGAAACCTGAATCAGGCTGTCTTGATAATATCAAGTCATGGG
TTATAGGTGCAATAGCGCTGCTCTGCCTATTAGGATTGACCTGGGCCTTT
GGACTCATGTATATTAATGAAAGCACAGTCATCATGGCGTATCTCTTCAC
CATTTTCAATTCTCTACAGGGGATGTTTATATTCATTTTCCACTGTGTCC
TACAGAAGAAGGTACGGAAAGAGTATGGGAAATGCCTACGGACGCATTGC
TGTAGTGGGAAAAGCACGGAGAGTTCGATTGGCTCAGGGAAAACATCTGG
TTCTCGAACTCCAGGACGGTATTCCACAGGCTCACAGAGCCGGATTCGGA
GAATGTGGAATGACACCGTCCGAAAGCAGTCAGAGTCATCCTTCATCACT
GGAGACATAAACAGCTCAGCGTCGCTCAACAGAGAGGGGCTTCTGAACAA
TGCCAGGGATACAAGTGTCATGGATACTCTACCACTGAATGGTAACCATG
GCAACAGTTACAGCATTGCTGGCGGCGAATACCTGAGCAACTGTGTGCAA
ATTATAGACCGTGGCTATAACCACAACGAGACCGCCCTAGAAAAAAAGAT
CCTAAAGGAACTCACTTCCAACTATATCCCTTCATACCTGAACAACCACG
AGCGCTCCAGCGAACAGAACCGGAACATGATGAACAAACTGGTGGACAAC
TTAGGCAGTGGGAGTGAAGATGACGCCATAGTCCTGGATGACGCAGCGTC
CTTTAACCACGAGGAGAGTCTGGGCCTGGAACTCATTCACGAGGAATCGG
ATGCTCCCTTGCTGCCCCCGAGGGTTTACTCCACCGATAACCACCAGCCA
CACCATTACAGCAGGAGGCGGCTCCCCCAGGACCACAGCGAGAGCTTCTT
CCCTCTGCTAACCGACGAGCACACAGAAGACCCGCAGTCACCGCACAGGG
ACTCTCTGTACACCAGCATGCCGGCCCTGGCCGGTGTGCCCGCTGCAGAC
AGTGTGACCACCAGCACCCAGACCGAAGCCGCAGCGGCCAAGGGTGGTGA
CGCCGAAGATGTTTACTACAAAAGCATGCCAAACCTGGGCTCCAGAAACC
ATGTGCACCCGCTGCACGCCTACTACCAGCTAGGGCGAGGCAGCAGCGAT
GGATTCATAGTTCCTCCCAATAAAGATGGGGCCTCTCCGGAGGGGACTTC
CAAAGGACCGGCGCACTTGGTCACTAGTCTATAG

Figure 6f
Peptide sequences for YSG2 (SEQ ID No.12)( CIRL, rat )
CIRL-3 variant BA

MCPPQLFILMMLLAPVVHGGKHNERHPALAAPLRHAEHSPGGPLPPRHLL
QQPAAERSTAHRGQGPRGTARGVRGPGAPGAQIAAQAFSRAPIPMAVVRR
ELSCESYPIELRCPGTDVIMIESANYGRTDDKICDSDPAQMENIRCYLPD
AYKIMSQRCNNRTQCAVVAGPDVFPDPCPGTYKYLEVQYECVPYKVEQKV
FLCPGLLKGVYQSEHLFESDHQSGAWCKDPLQASDKIYYMPWTPYRTDTL
TEYSSKDDFIAGRPTTTYKLPHRVDGTGFVVYDGALFFNKERTRNIVKFD
LRTRIKSGEAIIANANYHDTSPYRWGGKSDIDLAVDENGLWVIYATEQNN
GKIVISQLNPYTLRIEGTWDTAYDKRSASNAFMICGILYVVKSVYEDDDN
EATGNKIDYIYNTDQSKDSLVDVPFPNSYQYIAAVDYNPRDNLLYVWNNY
HVVKYSLDFGPLDSRSGPVHHGQVSYISPPIHLDSDLERPPVRGISTTGP
LGMGSTTTSTTLRTTTWNLGRSTTPSLPGRRNRSTSTPSPAIEVLDVTTH
LPSAASQIPAMEESCEAVEAREIMWFKTRQGQVAKQSCPAGTIGVSTYLC
LAPDGIWDPQGPDLSNCSSPWVNHITQKLKSGETAANIARELAEQTRNHL
NAGDITYSVRAMDQLVGLLDVQLRNLTPGGKDSAARSLNKLQKRERSCRA
YVQAMVETVNNLLQPQALNAWRDLTTSDQLRAATMLLDTVEESAFVLADN
LLKTDIVRENTDNIQLEVARLSTEGNLEDLKFPENTGHGSTIQLSANTLK
QNGRNGEIRVAFVLYNNLGPYLSTENASMKLGTEAMSTNHSVIVNSPVIT
AAINKEFSNKVYLADPVVFTVKHIKQSEENFNPNCSFWSYSKRTMTGYWS
TQGCRLLTTNKTHTTCSCNHLTNFAVLMAHVEVKHSDAVHDLLLDVITWV
GILLSLVCLLICIFTFCFFRGLQSDRNTIHKNLCISLFVAELLFLIGINR
TDQPIACAVFAALLHFFFLAAFTWMFLEGVQLYIMLVEVFESEHSRRKYF
YLVGYGMPALIVAVSAAVDYRSYGTDKVCWLRLDTYFIWSFIGPATLIIM
LNVIFLGIALYKMFHHTAILKPESGCLDNIKSWVIGAIALLCLLGLTWAF
GLMYINESTVIMAYLFTIFNSLQGMFIFIFHCVLQKKVRKEYGKCLRTHC
CSGKSTESSIGSGKTSGSRTPGRYSTGSQSRIRRMWNDTVRKQSESSFIT
GDINSSASLNREGLLNNARDTSVMDTLPLNGNHGNSYSIAGGEYLSNCVQ
IIDRGYNHNETALEKKILKELTSNYIPSYLNNHERSSEQNRNMMNKLVDN
LGSGSEDDAIVLDDAASFNHEESLGLELIHEESDAPLLPPRVYSTDNHQP
HHYSRRRLPQDHSESFFPLLTDEHTEDPQSPHRDSLYTSMPALAGVPAAD
SVTTSTQTEAAAAKGGDAEDVYYKSMPNLGSRNHVHPLHAYYQLGRGSSD
GFIVPPNKDGASPEGTSKGPAHLVTSL

FIGURE 7a
Gene sequence for YSG 5 (SEQ ID No.13)( TRK E, human)

GAGAGATGCTGCCCCCACCCCCTTAGGCCCGAGGGATCAGGAGCTATGGGACCAGAGGCC
CTGTCATCTTTACTGCTGCTGCTCTTGGTGGCAAGTGGAGATGCTGACATGAAGGGACAT
TTTGATCCTGCCAAGTGCCGCTATGCCCTGGGCATGCAGGACCGGACCATCCCAGACAGT
GACATCTCTGCTTCCAGCTCCTGGTCAGATTCCACTGCCGCCCGCCACAGCAGGTTGGAG
AGCAGTGACGGGGATGGGGCCTGGTGCCCCGCAGGGTCGGTGTTTCCCAAGGAGGAGGAG
TACTTGCAGGTGGATCTACAACGACTCCACCTGGTGGCTCTGGTGGGCACCCAGGGACGG
CATGCCGGGGGCCTGGGCAAGGAGTTCTCCCGGAGCTACCGGCTGCGTTACTCCCGGGAT
GGTCGCCGCTGGATGGGCTGGAAGGACCGCTGGGGTCAGGAGGTGATCTCAGGCAATGAG
GACCCTGAGGGAGTGGTGCTGAAGGACCTTGGGCCCCCCATGGTTGCCCGACTGGTTCGC

TTCTACCCCCGGGCTGACCGGGTCATGAGTGTCTGTCTGCGGGTAGAGCTCTATGGCTGC
CTCTGGAGGGATGGACTCCTGTCTTACACCGCCCCTGTGGGGCAGACAATGTATTTATCT
GAGGCCGTGTACCTCAACGACTCCACCTATGACGGACATACCGTGGGCGGACTGCAGTAT
GGGGGTCTGGGCCAGCTGGCAGATGGTGTGGTGGGGCTGGATGACTTTAGGAAGAGTCAG
GAGCTGCGGGTCTGGCCAGGCTATGACTATGTGGGATGGAGCAACCACAGCTTCTCCAGT
GGCTATGTGGAGATGGAGTTTGAGTTTGACCGGCTGAGGGCCTTCCAGGCTATGCAGGTC
CACTGTAACAACATGCACACGCTGGGAGCCCGTCTGCCTGGCGGGGTGGAATGTCGCTTC
CGGCGTGGCCCTGCCATGGCCTGGGAGGGGGAGCCCATGCGCCACAACCTAGGGGGCAAC
CTGGGGGACCCCAGAGCCCGGGCTGTCTCAGTGCCCCTTGGCGGCCGTGTGGCTCGCTTT
CTGCAGTGCCGCTTCCTCTTTGCGGGGCCCTGGTTACTCTTCAGCGAAATCTCCTTCATC
TCTGATGTGGTGAACAATTCCTCTCCGGCACTGGGAGGCACCTTCCCGCCAGCCCCCTGG
TGGCCGCCTGGCCCACCTCCCACCAACTTCAGCAGCTTGGAGCTGGAGCCCAGAGGCCAG
CAGCCCGTGGCCAAGGCCGAGGGGAGCCCGACCGCCATCCTCATCGGCTGCCTGGTGGCC
ATCATCCTGCTCCTGCTGCTCATCATTGCCCTCATGCTCTGGCGGCTGCACTGGCGCAGG
CTCCTCAGCAAGGCTGAACGGAGGGTGTTGGAAGAGGAGCTGACGGTTCACCTCTCTGTC
CCTGGGGACACTATCCTCATCAACAACCGCCCAGGTCCTAGAGAGCCACCCCCGTACCAG
GAGCCCCGGCCTCGTGGGAATCCGCCCCACTCCGCTCCCTGTGTCCCCAATGGCTCTGCC
TACAGTGGGGACTATATGGAGCCTGAGAAGCCAGGCGCCCCGCTTCTGCCCCCACCTCCC
CAGAACAGCGTCCCCCATTATGCCGAGGCTGACATTGTTACCCTGCAGGGCGTCACCGGG
GGCAACACCTATGCTGTGCCTGCACTGCCCCCAGGGGCAGTCGGGGATGGGCCCCCCAGA
GTGGATTTCCCTCGATCTCGACTCCGCTTCAAGGAGAAGCTTGGCGAGGGCCAGTTTGGG
GAGGTGCACCTGTGTGAGGTCGACAGCCCTCAAGATCTGGTCAGTCTTGATTTCCCCCTT
AATGTGCGTAAGGGACACCCTTTGCTGGTAGCTGTCAAGATCTTACGGCCAGATGCCACC
AAGAATGCCAGGAATGATTTCCTGAAAGAGGTGAAGATCATGTCGAGGCTCAAGGACCCA
AACATCATTCGGCTGCTGGGCGTGTGTGTGCAGGACGACCCCCTCTGCATGATTACTGAC
TACATGGAGAACGGCGACCTCAACCAGTTCCTCAGTGCCCACCAGCTGGAGGACAAGGCA
GCCGAGGGGGCCCCTGGGGACGGGCAGGCTGCGCAGGGGCCCACCATCAGCTACCCAATG
CTGCTGCATGTGGCAGCCCAGATCGCCTCCGGCATGCGCTATCTAGCCACACTCAACTTT
GTACATCGGGACCTGGCCACGCGGAACTGCCTAGTTGGGGAAAATTTCACCATCAAAATC
GCAGACTTTGGCATGAGCCGGAACCTCTATGCTGGGGACTATTACCGTGTGCAGGGCCGG
GCAGTGCTGCCCATCCGCTGGATGGCCTGGGAGTGCATCCTCATGGGGAAGTTCACGACT
GCGAGTGACGTGTGGGCCTTTGGTGTGACCCTGTGGGAGGTGCTGATGCTCTGTAGGGCC
CAGCCCTTTGGGCAGCTCACCGACGAGCAGGTCATCGAGAACGCGGGGGAGTTCTTCCGG
GACCAGGGCCGGCAGGTGTACCTGTCCCGGCCGCCTGCCTGCCCGCAGGGCCTATATGAG
CTGATGCTTCGGTGCTGGAGCCGGGAGTCTGAGCAGCGACCACCCTTTTCCCAGCTGCAT
CGGTTCCTGGCAGAGGATGCACTCAACACGGTGTGAATCACACATCCAGCTGCCCCTCCC
TCAGGGAGTGATCCAGGGGAAGCCAGTGACACTAAAACAAGAGGACACAATGGCACCTCT
GCCCTTCCCCTCCCGACAGCCCATCACCTCTAATAGAGGCAGTGAGACTGCAGGTGGGCT
GGGCCCACCCAGGGAGCTGATGCCCCTTCTCCCCTTCCTGGACACACTCTCATGTCCCCT
TCCTGTTCTTCCTTCCTAGAAGCCCCTGTCGCCCACCCAGCTGGTCCTGTGGATGGGATC
CTCTCCACCCTCCTCTAGCCATCCCTTGGGGAAGGGTGGGGAGAAATATAGGATAGACAC
TGGACATGGCCCATTGGAGCACCTGGGCCCCACTGGACAACACTGATTCCTGGAGAGGTG
GCTGCGCCCCCAGCTTCTCTCTCCCTGTCACACACTGGACCCCACTGGCTGAGAATCTGG
GGGTGAGGAGGACAAGAAGGAGAGGAAAATGTTTCCTTGTGCCTGCTCCTGTACTTGTCC
TCAGCTTGGGCTTCTTCCTCCTCCATCACCTGAAACACTGGACCTGGGGGTAGCCCCGCC
CCAGCCCTCAGTCACCCCCACTTCCCACCTGCAGTCTTGTAGCTAGAACTTCTCTAAGCC
TATACGTTTCTGTGGAGTAAATATTGGGATTGGGGGGAAAGAGGGAGCAACGGCCCATAG
CCTTGGGGTTGGACATCTCTAGTGTAGCTGCCACATTGATTTTTCTATAATCACTTGGGG
TTTGTACATTTTTGGGGGGAGAGACACAGATTTTTACACTAATATATGGACCTAGCTTGA

GGCAATTTTAATCCCCTGCACTAGGCAGGTAATAATAAAGGTTGAGTTTTCCACAAAAAA
AAAAAAAAAAAAAA

Figure 7b
Peptide sequence for YSG 5 (SEQ ID No.14)(TRK E, human)

MGPEALSSLLLLLLLVASGDADMKGHFDPAKCRYALGMQDRTIPDSDISASSSWSDSTAAR
HSRLESSDGDGAWCPAGSVFPKEEEYLQVDLQRLHLVALVGTQGRHAGGLGKEFSRSYRL
RYSRDGRRWMGWKDRWGQEVISGNEDPEGVVLKDLGPPMVARLVRFYPRADRVMSVCLRV
ELYGCLWRDGLLSYTAPVGQTMYLSEAVYLNDSTYDGHTVGGLQYGGLGQLADGVVGLDD
FRKSQELRVWPGYDYVGWSNHSFSSGYVEMEFEFDRLRAFQAMQVHCNNMHTLGARLPGG
VECRFRRGPAMAWEGEPMRHNLGGNLGDPRARAVSVPLGGRVARFLQCRFLFAGPWLLFS
EISFISDVVNNSSPALGGTFPPAPWWPPGPPPTNFSSLELEPRGQQPVAKAEGSPTAILI
GCLVAIILLLLLIIALMLWRLHWRRLLSKAERRVLEEELTVHLSVPGDTILINNRPGPRE
PPPYQEPRPRGNPPHSAPCVPNGSAYSGDYMEPEKPGAPLLPPPPQNSVPHYAEADIVTL
QGVTGGNTYAVPALPPGAVGDGPPRVDFPRSRLRFKEKLGEGQFGEVHLCEVDSPQDLVS
LDFPLNVRKGHPLLVAVKILRPDATKNARNDFLKEVKIMSRLKDPNIIRLLGVCVQDDPL
CMITDYMENGDLNQFLSAHQLEDKAAEGAPGDGQAAQGPTISYPMLLHVAAQIASGMRYL
ATLNFVHRDLATRNCLVGENFTIKIADFGMSRNLYAGDYYRVQGRAVLPIRWMAWECILM
GKFTTASDVWAFGVTLWEVLMLCRAQPFGQLTDEQVIENAGEFFRDQGRQVYLSRPPACP
QGLYELMLRCWSRESEQRPPFSQLHRFLAEDALNTV

FIGURE 8a
Gene sequence for YSG7 (SEQ ID No.15)( UNC5H1, rat)

ATGGCCGTCCGGCCCGGCCTGTGGCCAGTGCTCCTGGGCATAGTCCTCGCCGCCTGGCTT
CGTGGTTCGGGTGCCCAGCAGAGTGCCACGGTGGCCAATCCAGTGCCCGGTGCCAACCCC
GACCTGCTGCCCCACTTCCTGGTAGAGCCTGAGGACGTGTACATTGTCAAGAACAAGCCG
GTGTTGTTGGTGTGCAAGGCTGTGCCTGCCACCCAGATCTTCTTCAAGTGCAATGGGGAA
TGGGTCCGCCAGGTCGATCACGTAATTGAACGCAGCACCGACAGCAGCAGCGGATTGCCA
ACCATGGAGGTCCGTATCAACGTATCGAGGCAGCAGGTAGAGAAAGTGTTTGGGCTGGAG
GAATACTGGTGCCAGTGTGTGGCATGGAGCTCCTCGGGTACCACCAAAAGTCAGAAGGCC
TACATCCGGATTGCCTATTTGCGCAAGAACTTTGAGCAGGAGCCACTGGCCAAGGAAGTG
TCACTGGAGCAAGGCATTGTACTACCTTGTCGCCCCCCAGAAGGAATCCCCCCAGCTGAG
GTGGAGTGGCTTCGAAATGAGGACCTCGTGGACCCCTCCCTCGATCCCAATGTGTACATC
ACGCGGGAGCACAGCCTAGTCGTGCGTCAGGCCCGCCTGGCCGACACGGCCAACTACACC
TGTGTGGCCAAGAACATCGTAGCCCGTCGCCGAAGCACCTCTGCAGCGGTCATTGTTTAT
GTGAACGGTGGGTGGTCGACGTGGACTGAGTGGTCCGTCTGCAGCGCCAGCTGTGGGCGT
GGCTGGCAGAAACGGAGCCGGAGCTGCACCAACCCGGCACCTCTCAACGGGGGCGCCTTC
TGTGAGGGGCAGAATGTCCAGAAAACAGCCTGCGCCACTCTGTGCCCAGTGGATGGGAGC
TGGAGTTCGTGGAGTAAGTGGTCAGCCTGTGGGCTTGACTGCACCCACTGGCGGAGCCGC
GAGTGCTCTGACCCAGCACCCCGCAATGGAGGTGAGGAGTGTCGGGGTGCTGACCTGGAC
ACCCGCAACTGTACCAGTGACCTCTGCCTGCACACCGCTTCTTGCCCCGAGGACGTGGCT
CTCTACATCGGCCTTGTCGCTGTGGCTGTGTGCCTCTTCTTGCTGTTGCTGGCCCTTGGA
CTCATTTACTGTCGCAAGAAGGAAGGGCTGGACTCCGATGTGGCCGACTCGTCCATCCTC
ACCTCGGGCTTCCAGCCTGTCAGCATCAAGCCCAGCAAAGCAGACAACCCCCACCTGCTC
ACCATCCAGCCAGACCTCAGCACCACCACTACCACCTACCAGGGCAGTCTATGTTCGAGG

CAGGATGGACCCAGCCCCAAGTTCCAGCTCTCTAATGGTCACCTGCTCAGCCCACTGGGG
AGTGGCCGCCATACGTTGCACCACAGCTCACCCACCTCTGAGGCTGAGGACTTCGTCTCC
CGCCTCTCCACCCAAAACTACTTTCGTTCCCTGCCCCGCGGCACCAGCAACATGGCCTAC
GGGACCTTCAACTTCCTCGGGGGCCGGCTGATGATCCCTAATACGGGGATCAGCCTCCTC
ATACCCCCGGATGCCATCCCCCGAGGAAAGATCTACGAGATCTACCTCACACTGCACAAG
CCAGAAGACGTGAGGTTGCCCCTAGCTGGCTGTCAGACCCTGCTGAGTCCAGTCGTTAGC
TGTGGGCCCCCAGGAGTCCTGCTCACCCGGCCAGTCATCCTTGCAATGGACCACTGTGGA
GAGCCCAGCCCTGACAGCTGGAGTCTGCGCCTCAAAAAGCAGTCCTGCGAGGGCAGTTGG
GAGGATGTGCTGCACCTTGGTGAGGAGTCACCTTCCCACCTCTACTACTGCCAGCTGGAG
GCCGGGGCCTGCTATGTCTTCACGGAGCAGCTGGGCCGCTTTGCCCTGGTAGGAGAGGCC
CTCAGCGTGGCTGCCACCAAGCGCCTCAGGCTCCTTCTGTTTGCTCCCGTGGCCTGTACG
TCCCTTGAGTACAACATCCGAGTGTACTGCCTACACGACACCCACGACGCTCTCAAGGAG
GTGGTGCAGCTGGAGAAGCAGCTAGGTGGACAGCTGATCCAGGAGCCTCGCGTCCTGCAC
TTCAAAGACAGTTACCACAACCTACGTCTCTCCATCCACGACGTGCCCAGCTCCCTGTGG
AAGAGCAAGCTACTTGTCAGCTACCAGGAGATCCCTTTTTACCACATCTGGAACGGCACC
CAGCAGTATCTGCACTGCACCTTCACCCTGGAGCGCATCAACGCCAGCACCAGCGACCTG
GCCTGCAAGGTGTGGGTGTGGCAGGTGGAGGGAGATGGGCAGAGCTTCAACATCAACTTC
AACATCACTAAGGACACAAGGTTTGCTGAATTGTTGGCTCTGGAGAGTGAAGGGGGGGTC
CCAGCCCTGGTGGGCCCCAGTGCCTTCAAGATCCCCTTCCTCATTCGGCAAAAGATCATC
GCCAGTCTGGACCCACCCTGCAGCCGGGGCGCCGACTGGAGAACTCTAGCCCAGAAACTT
CACCTGGACAGCCATCTTAGCTTCTTTGCCTCCAAGCCCAGCCCTACAGCCATGATCCTC
AACCTATGGGAGGCACGGCACTTCCCCAACGGCAACCTCGGCCAGCTGGCAGCAGCTGTG
GCCGGACTGGGCCAACCAGATGCTGGCCTCTTCACGGTGTCGGAGGCCGAGTGTTGA

Figure 8b
Peptide sequence for YSG7 (SEQ ID No.16)( UNC5H1, rat)

MAVRPGLWPVLLGIVLAAWLRGSGAQQSATVANPVPGANPDLLPHFLVEPEDVYIVKNKP
VLLVCKAVPATQIFFKCNGEWVRQVDHVIERSTDSSSGLPTMEVRINVSRQQVEKVFGLE
EYWCQCVAWSSSGTTKSQKAYIRIAYLRKNFEQEPLAKEVSLEQGIVLPCRPPEGIPPAE
VEWLRNEDLVDPSLDPNVYITREHSLVVRQARLADTANYTCVAKNIVARRRSTSAAVIVY
VNGGWSTWTEWSVCSASCGRGWQKRSRSCTNPAPLNGGAFCEGQNVQKTACATLCPVDGS
WSSWSKWSACGLDCTHWRSRECSDPAPRNGGEECRGADLDTRNCTSDLCLHTASCPEDVA
LYIGLVAVAVCLFLLLLALGLIYCRKKEGLDSDVADSSILTSGFQPVSIKPSKADNPHLL
TIQPDLSTTTTTYQGSLCSRQDGPSPKFQLSNGHLLSPLGSGRHTLHHSSPTSEAEDFVS
RLSTQNYFRSLPRGTSNMAYGTFNFLGGRLMIPNTGISLLIPPDAIPRGKIYEIYLTLHK
PEDVRLPLAGCQTLLSPVVSCGPPGVLLTRPVILAMDHCGEPSPDSWSLRLKKQSCEGSW
EDVLHLGEESPSHLYYCQLEAGACYVFTEQLGRFALVGEALSVAATKRLRLLLFAPVACT
SLEYNIRVYCLHDTHDALKEVVQLEKQLGGQLIQEPRVLHFKDSYHNLRLSIHDVPSSLW
KSKLLVSYQEIPFYHIWNGTQQYLHCTFTLERINASTSDLACKVWVWQVEGDGQSFNINF
NITKDTRFAELLALESEGGVPALVGPSAFKIPFLIRQKIIASLDPPCSRGADWRTLAQKL
HLDSHLSFFASKPSPTAMILNLWEARHFPNGNLGQLAAAVAGLGQPDAGLFTVSEAEC

FIGURE 9a

Gene sequences for YSG8 (SEQ ID No.17)
(synapsin I, rat) Synapsin IA

ATGAACTACCTGCGGCGCCGCCTGTCGGACAGCAACTTCATGGCCAATCT
GCCTAATGGGTATATGACAGACCTGCAGCGCCCGCAACCACCTCCGCCGC
CGCCCTCAGCCGCCAGCCCAGGGGCCACTCCCGGATCCGCTGCTGCCTCT
GCTGAGAGGGCCTCCACAGCTGCTCCAGTGGCCTCTCCAGCAGCCCCTAG
TCCCGGGTCCTCGGGGGGCGGTGGCTTCTTCTCCTCGCTGTCTAACGCGG
TCAAACAGACCACAGCAGCTGCAGCCGCCACCTTCAGTGAGCAGGTGGGC
GGTGGCTCTGGGGGCGCAGGCCGCGGGGGCGCCGCCGCCAGGGTGCTGCT
GGTCATCGACGAGCCGCACACCGACTGGGCAAAATACTTCAAAGGGAAGA
AGATCCATGGAGAAATTGACATTAAAGTAGAGCAAGCTGAATTCTCCGAT
CTCAATCTTGTGGCTCATGCCAATGGTGGATTCTCCGTGGACATGGAAGT
TCTTCGGAATGGGGTCAAAGTTGTGAGGTCTCTGAAGCCAGACTTTGTGC
TGATCCGCCAGCATGCCTTCAGCATGGCACGTAATGGAGACTACCGCAGT
TTGGTCATTGGGCTGCAGTATGCTGGGATCCCCAGTGTTAACTCTTTGCA
TTCTGTCTACAACTTTTGTGACAAACCCTGGGTGTTTGCCCAGATGGTTC
GACTACACAAGAAGCTTGGAACAGAGGAATTCCCTCTGATTGATCAGACT
TTCTATCCCAATCATAAAGAGATGCTCAGCAGCACAACATACCCTGTAGT
TGTGAAGATGGGCCACGCACACTCTGGGATGGGCAAGGTCAAGGTAGACA
ACCAACATGACTTCCAGGATATTGCAAGTGTTGTGGCACTGACTAAGACA
TATGCCACTGCTGAGCCCTTCATTGATGCTAAATACGATGTGCGTGTCCA
GAAGATTGGGCAGAACTACAAGGCCTACATGAGGACATCAGTGTCAGGGA
ACTGGAAGACCAATACAGGCTCTGCTATGCTTGAGCAGATTGCTATGTCT
GACAGGTACAAGTTGTGGGTAGACACGTGCTCAGAGATTTTTGGGGGACT
TGACATCTGCGCAGTGGAAGCACTGCATGGCAAGGACGGAAGGGATCACA
TTATTGAGGTGGTGGGCTCCTCCATGCCACTCATTGGGGATCACCAGGAT
GAAGACAAGCAGCTCATCGTGGAACTTGTGGTCAACAAGATGACTCAGGC
TCTGCCTCGGCAGCGGGATGCTTCCCCTGGCAGGGGTTCCCACAGCCAGA
CTCCATCCCCAGGAGCCCTGCCCTTGGGCCGCCAGACCTCCCAGCAGCCT
GCAGGACCTCCTGCTCAACAACGACCCCCACCCCAGGGAGGCCCTCCACA
ACCAGGCCCAGGACCTCAGCGCCAGGGACCCCCGCTGCAACAGCGCCCAC
CCCCACAAGGCCAGCAACATCTTTCTGGCCTTGGACCCCCAGCTGGCAGC
CCTCTGCCCCAGCGCCTACCAAGTCCCACAGCAGCACCTCAGCAGTCCGC
CTCTCAGGCCACACCAATGACCCAGGGTCAAGGCCGCCAGTCACGGCCAG
TGGCAGGAGGCCCCGGAGCACCTCCAGCAGCCCGCCCGCCGGCCTCCCCA
TCTCCACAGCGTCAGGCAGGGCCCCCACAGGCTACCCGTCAGGCATCTAT
CTCTGGTCCAGCTCCACCGAAGGTCTCAGGAGCCTCACCCGGAGGGCAGC
AGCGCCAAGGCCCTCCCCAGAAACCCCCAGGCCCTGCTGGTCCCATTCGT
CAGGCCAGCCAGGCAGGTCCCGGACCTCGCACTGGGCCACCCACCACACA
GCAGCCCCGGCCCAGCGGCCCAGGTCCTGCTGGACGTCCCACCAAACCAC
AGCTGGCTCAGAAACCCAGCCAGGATGTGCCACCACCCATCATTGCTGCT
GCCGGGGGACCCCCGCACCCCCAGCTCAACAAATCCCAGTCTCTGACCAA
TGCCTTCAACCTTCCAGAGCCAGCCCCGCCCAGGCCCAGCCTTAGCCAGG
ATGAGGTGAAAGCTGAGACCATCCGCAGCCTGAGGAAGTCTTTCGCCAGC
CTCTTCTCCGACTGA

Figure 9b
Peptide sequence for YSG8 (SEQ ID No.18)
(synapsin I, rat) Synapsin IA

MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPPSAASPGATPGSAAAS
AERASTAAPVASPAAPSPGSSGGGGFFSSLSNAVKQTTAAAAATFSEQVG
GGSGGAGRGGAAARVLLVIDEPHTDWAKYFKGKKIHGEIDIKVEQAEFSD
LNLVAHANGGFSVDMEVLRNGVKVVRSLKPDFVLIRQHAFSMARNGDYRS
LVIGLQYAGIPSVNSLHSVYNFCDKPWVFAQMVRLHKKLGTEEFPLIDQT
FYPNHKEMLSSTTYPVVVKMGHAHSGMGKVKVDNQHDFQDIASVVALTKT
YATAEPFIDAKYDVRVQKIGQNYKAYMRTSVSGNWKTNTGSAMLEQIAMS
DRYKLWVDTCSEIFGGLDICAVEALHGKDGRDHIIEVVGSSMPLIGDHQD
EDKQLIVELVVNKMTQALPRQRDASPGRGSHSQTPSPGALPLGRQTSQQP
AGPPAQQRPPPQGGPPQPGPGPQRQGPPLQQRPPPQGQQHLSGLGPPAGS
PLPQRLPSPTAAPQQSASQATPMTQGQGRQSRPVAGGPGAPPAARPPASP
SPQRQAGPPQATRQASISGPAPPKVSGASPGGQQRQGPPQKPPGPAGPIR
QASQAGPGPRTGPPTTQQPRPSGPGPAGRPTKPQLAQKPSQDVPPPIIAA
AGGPPHPQLNKSQSLTNAFNLPEPAPPRPSLSQDEVKAETIRSLRKSFAS
LFSD

Figure 9c
Gene sequences for YSG8 (SEQ ID No.19)
(synapsin I, rat) Synapsin IB

ATGAACTACCTGCGGCGCCGCCTGTCGGACAGCAACTTCATGGCCAATCT
GCCTAATGGGTATATGACAGACCTGCAGCGCCCGCAACCACCTCCGCCGC
CGCCCTCAGCCGCCAGCCCAGGGGCCACTCCCGGATCCGCTGCTGCCTCT
GCTGAGAGGGCCTCCACAGCTGCTCCAGTGGCCTCTCCAGCAGCCCCTAG
TCCCGGGTCCTCGGGGGGCGGTGGCTTCTTCTCCTCGCTGTCTAACGCGG
TCAAACAGACCACAGCAGCTGCAGCCGCCACCTTCAGTGAGCAGGTGGGC
GGTGGCTCTGGGGGCGCAGGCCGCGGGGGCGCCGCCGCCAGGGTGCTGCT
GGTCATCGACGAGCCGCACACCGACTGGGCAAAATACTTCAAAGGGAAGA
AGATCCATGGAGAAATTGACATTAAAGTAGAGCAAGCTGAATTCTCCGAT
CTCAATCTTGTGGCTCATGCCAATGGTGGATTCTCCGTGGACATGGAAGT
TCTTCGGAATGGGGTCAAAGTTGTGAGGTCTCTGAAGCCAGACTTTGTGC
TGATCCGCCAGCATGCCTTCAGCATGGCACGTAATGGAGACTACCGCAGT
TTGGTCATTGGGCTGCAGTATGCTGGGATCCCCAGTGTTAACTCTTTGCA
TTCTGTCTACAACTTTTGTGACAAACCCTGGGTGTTTGCCCAGATGGTTC
GACTACACAAGAAGCTTGGAACAGAGGAATTCCCTCTGATTGATCAGACT
TTCTATCCCAATCATAAAGAGATGCTCAGCAGCACAACATACCCTGTAGT
TGTGAAGATGGGCCACGCACACTCTGGGATGGGCAAGGTCAAGGTAGACA
ACCAACATGACTTCCAGGATATTGCAAGTGTTGTGGCACTGACTAAGACA
TATGCCACTGCTGAGCCCTTCATTGATGCTAAATACGATGTGCGTGTCCA
GAAGATTGGGCAGAACTACAAGGCCTACATGAGGACATCAGTGTCAGGGA
ACTGGAAGACCAATACAGGCTCTGCTATGCTTGAGCAGATTGCTATGTCT
GACAGGTACAAGTTGTGGGTAGACACGTGCTCAGAGATTTTTGGGGGACT
TGACATCTGCGCAGTGGAAGCACTGCATGGCAAGGACGGAAGGGATCACA
TTATTGAGGTGGTGGGCTCCTCCATGCCACTCATTGGGGATCACCAGGAT

GAAGACAAGCAGCTCATCGTGGAACTTGTGGTCAACAAGATGACTCAGGC
TCTGCCTCGGCAGCGGGATGCTTCCCCTGGCAGGGGTTCCCACAGCCAGA
CTCCATCCCCAGGAGCCCTGCCCTTGGGCCGCCAGACCTCCCAGCAGCCT
GCAGGACCTCCTGCTCAACAACGACCCCCACCCCAGGGAGGCCCTCCACA
ACCAGGCCCAGGACCTCAGCGCCAGGGACCCCCGCTGCAACAGCGCCCAC
CCCCACAAGGCCAGCAACATCTTTCTGGCCTTGGACCCCCAGCTGGCAGC
CCTCTGCCCCAGCGCCTACCAAGTCCCACAGCAGCACCTCAGCAGTCCGC
CTCTCAGGCCACACCAATGACCCAGGGTCAAGGCCGCCAGTCACGGCCAG
TGGCAGGAGGCCCCGGAGCACCTCCAGCAGCCCGCCCGCCGGCCTCCCCA
TCTCCACAGCGTCAGGCAGGGCCCCCACAGGCTACCCGTCAGGCATCTAT
CTCTGGTCCAGCTCCACCGAAGGTCTCAGGAGCCTCACCCGGAGGGCAGC
AGCGCCAAGGCCCTCCCCAGAAACCCCCAGGCCCTGCTGGTCCCATTCGT
CAGGCCAGCCAGGCAGGTCCCGGACCTCGCACTGGGCCACCCACCACACA
GCAGCCCCGGCCCAGCGGCCCAGGTCCTGCTGGACGTCCCACCAAACCAC
AGCTGGCTCAGAAACCCAGCCAGGATGTGCCACCACCCATCATTGCTGCT
GCCGGGGGACCCCCGCACCCCCAGCTCAAAGCCAGCCCCGCCCAGGCCCA
GCCTTAG

Figure 9d
Peptide sequence for YSG8 (SEQ ID No.20)
(synapsin I, rat) Synapsin IB

MNYLRRRLSDSNFMANLPNGYMTDLQRPQPPPPPPSAASPGATPGSAAAS
AERASTAAPVASPAAPSPGSSGGGGFFSSLSNAVKQTTAAAAATFSEQVG
GGSGGAGRGGAAARVLLVIDEPHTDWAKYFKGKKIHGEIDIKVEQAEFSD
LNLVAHANGGFSVDMEVLRNGVKVVRSLKPDFVLIRQHAFSMARNGDYRS
LVIGLQYAGIPSVNSLHSVYNFCDKPWVFAQMVRLHKKLGTEEFPLIDQT
FYPNHKEMLSSTTYPVVVKMGHAHSGMGKVKVDNQHDFQDIASVVALTKT
YATAEPFIDAKYDVRVQKIGQNYKAYMRTSVSGNWKTNTGSAMLEQIAMS
DRYKLWVDTCSEIFGGLDICAVEALHGKDGRDHIIEVVGSSMPLIGDHQD
EDKQLIVELVVNKMTQALPRQRDASPGRGSHSQTPSPGALPLGRQTSQQP
AGPPAQQRPPPQGGPPQPGPGPQRQGPPLQQRPPPQGQQHLSGLGPPAGS
PLPQRLPSPTAAPQQSASQATPMTQGQGRQSRPVAGGPGAPPAARPPASP
SPQRQAGPPQATRQASISGPAPPKVSGASPGGQQRQGPPQKPPGPAGPIR
QASQAGPGPRTGPPTTQQPRPSGPGPAGRPTKPQLAQKPSQDVPPPIIAA
AGGPPHPQLKASPAQAQP

FIGURE 10a
Gene sequence for YSG10 (SEQ ID No. 21) (TNF-alpha, rat)

ATGAGCACAGAAAGCATGATCCGAGATGTGGAACTGGCAGAGGAGGCGCTCCCCAAAAAG
ATGGGGGGCCTCCAGAACTCCAGGCGGTGTCTGTGCCTCAGCCTCTTCTCATTCCTGCTC
GTGGCGGGGGCCACCACGCTCTTCTGTCTACTGAACTTCGGGGTGATCGGTCCCAACAAG
GAGGAGAAGTTCCCAAATGGGCTCCCTCTCATCAGTTCCATGGCCCAGACCCTCACACTC
AGATCATCTTCTCAAAACTCGAGTGACAAGCCCGTAGCCCACGTCGTAGCAAACCACCAA
GCAGAGGAGCAGCTGGAGTGGCTGAGCCAGCGTGCCAACGCCCTCCTGGCCAATGGCATG
GATCTCAAAGACAACCAACTGGTGGTACCAGCAGATGGGCTGTACCTTATCTACTCCCAG

GTTCTCTTCAAGGGACAAGGCTGCCCCGACTATGTGCTCCTCACCCACACCGTCAGCCGA
TTTGCCATTTCATACCAGGAGAAAGTCAGCCTCCTCTCCGCCATCAAGAGCCCTTGCCCT
AAGGACACCCCTGAGGGAGCTGAGCTCGAGCCCTGGTATGAGCCCATGTACCTGGGAGGA
GTCTTCCAGCTGGAGAAGGGGGACCTGCTCAGCGCTGAGGTCAACCTGCCCAAGTACTTA
GACATCACGGAGTCCGGGCAGGTCTACTTTGGAGTCATTGCTCTG

FIGURE 10b

Peptide sequence for YSG10 (SEQ ID No. 22) (TNF-alpha, rat)

MSTESMIRDVELAEEALPKKMGGLQNSRRCLCLSLFSFLLVAGATTLFCLLNFGVIGPNK
EEKFPNGLPLISSMAQTLTLRSSSQNSSDKPVAHVVANHQAEEQLEWLSQRANALLANGM
DLKDNQLVVPADGLYLIYSQVLFKGQGCPDYVLLTHTVSRFAISYQEKVSLLSAIKSPCP
KDTPEGAELKPWYEPMYLGGVFQLEKGDLLSAEVNLPKYLDITESGQVYFGVIAL

Figure 11

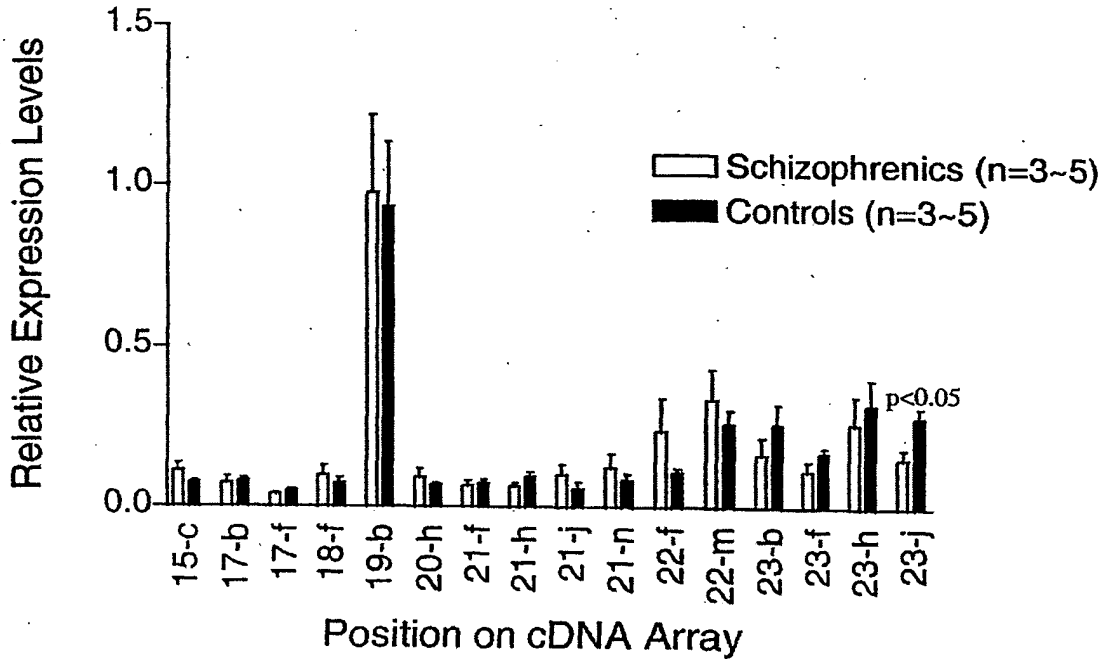


Figure 12

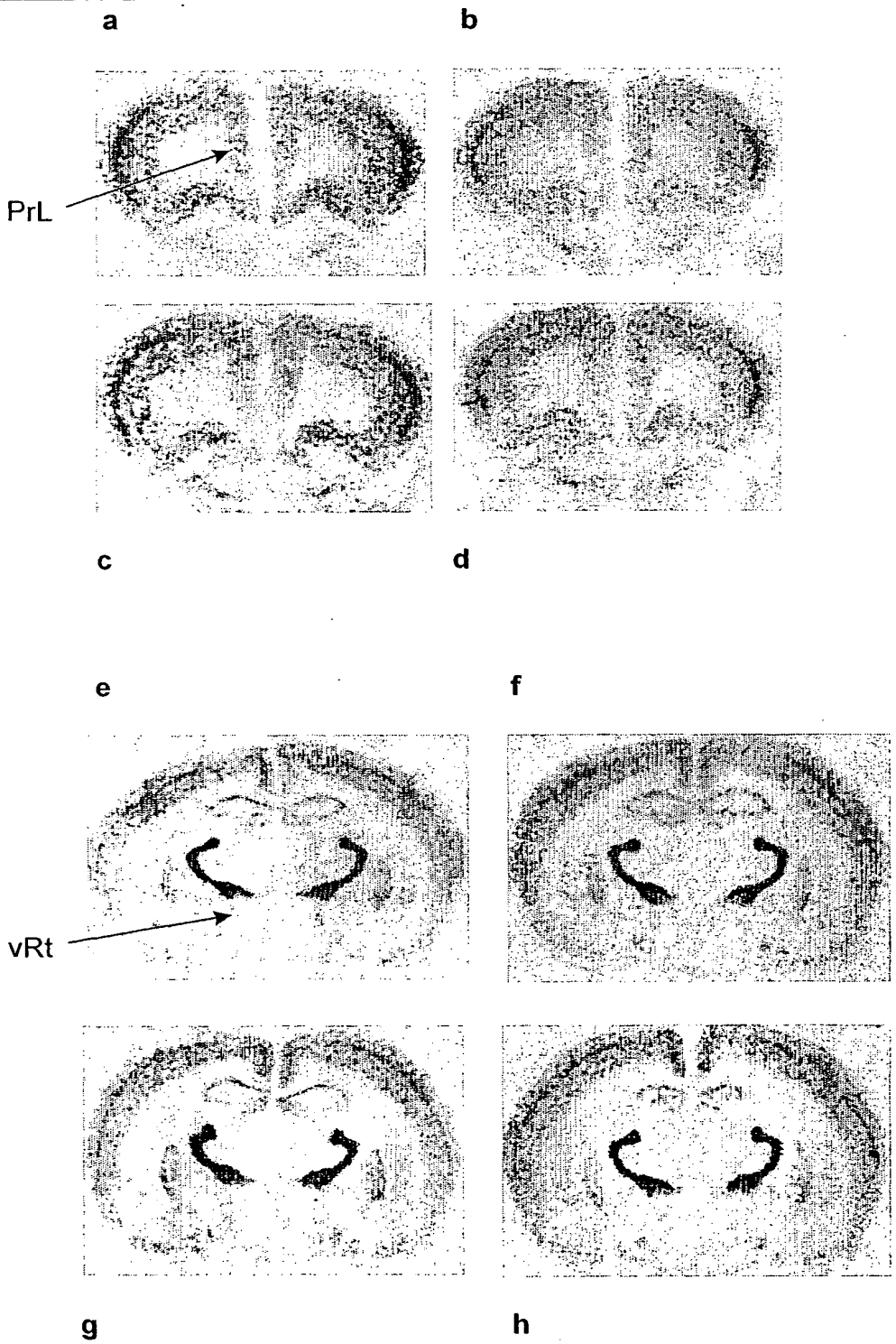


Figure 13

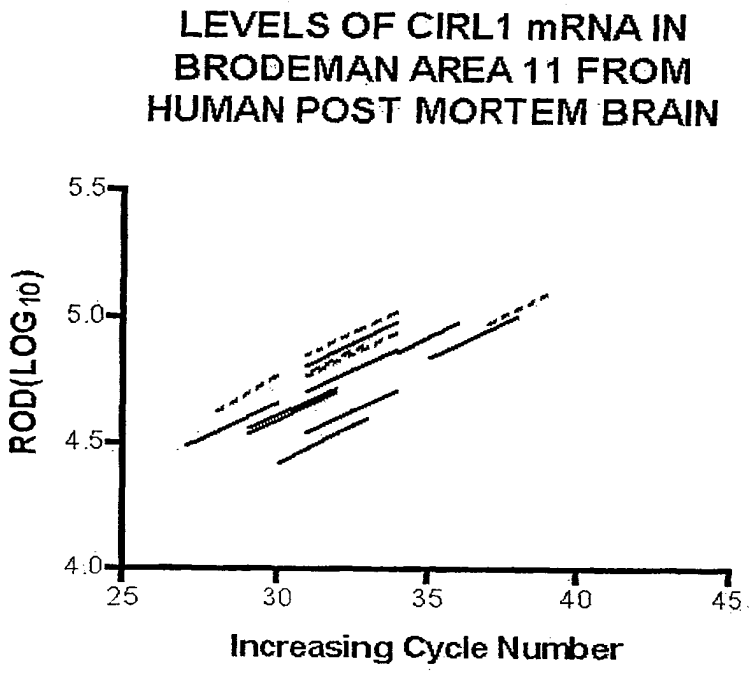



Figure 14

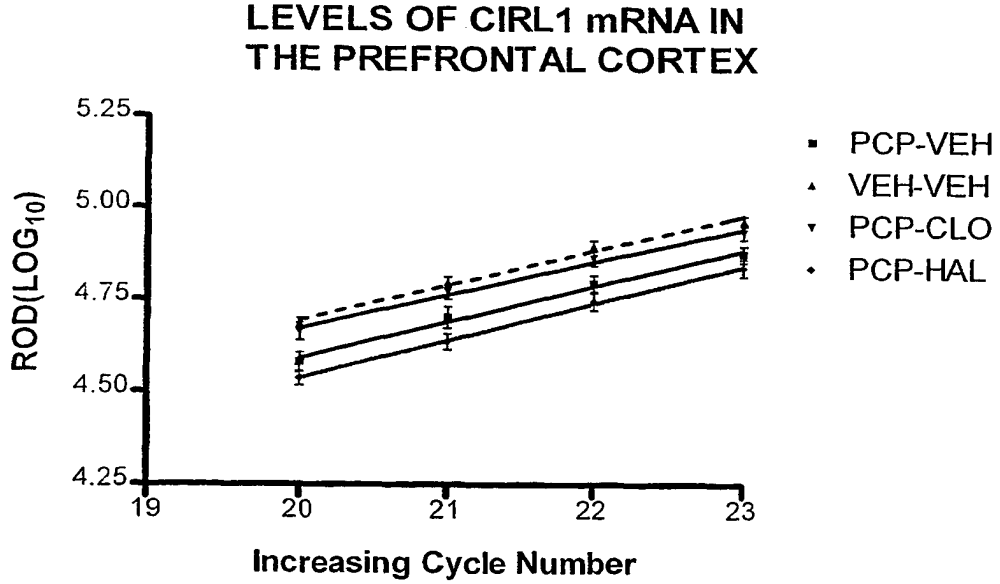


Figure 15

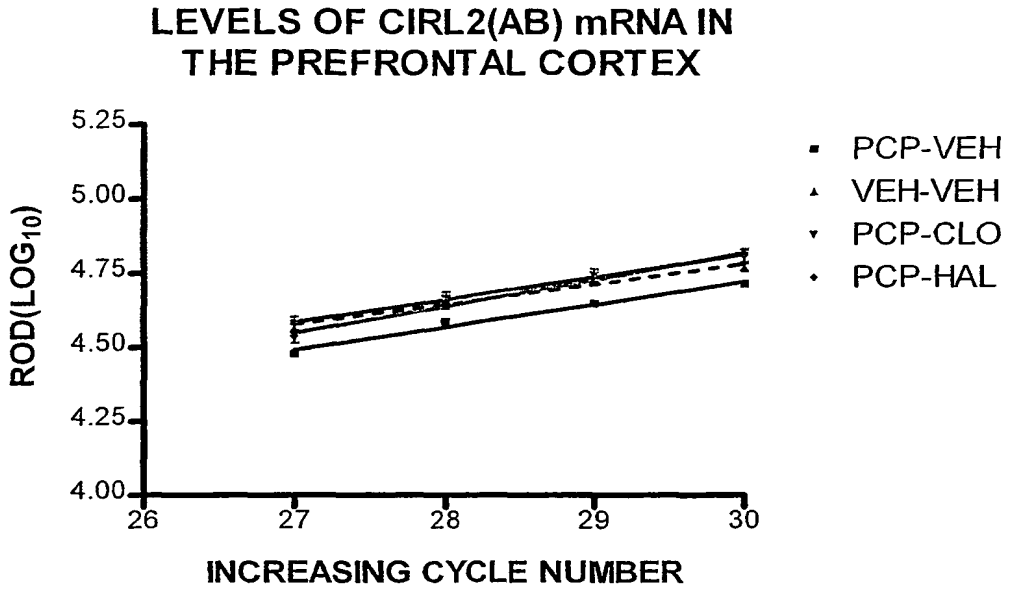



Figure 16

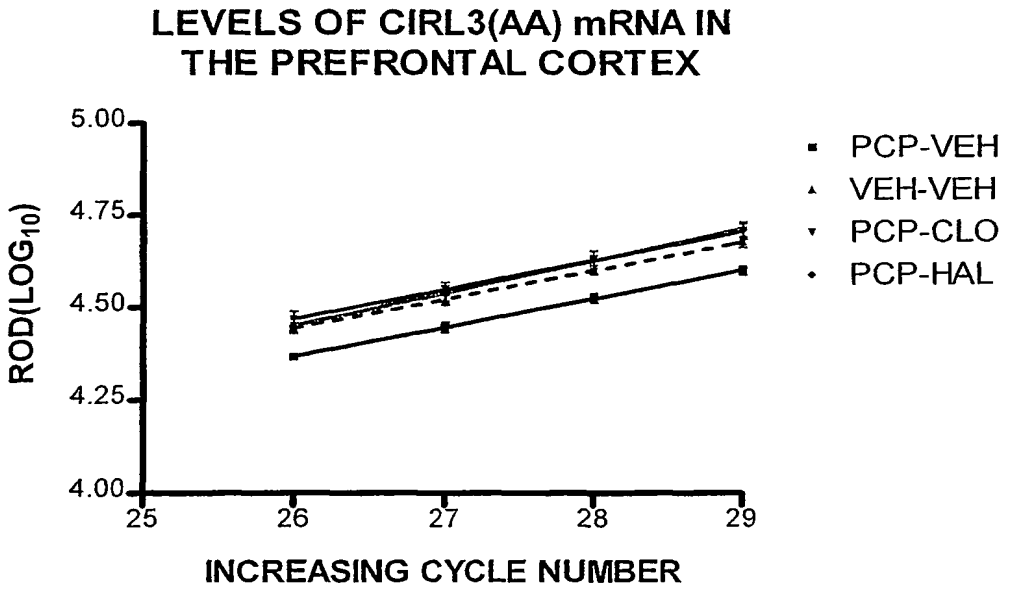


Figure 17

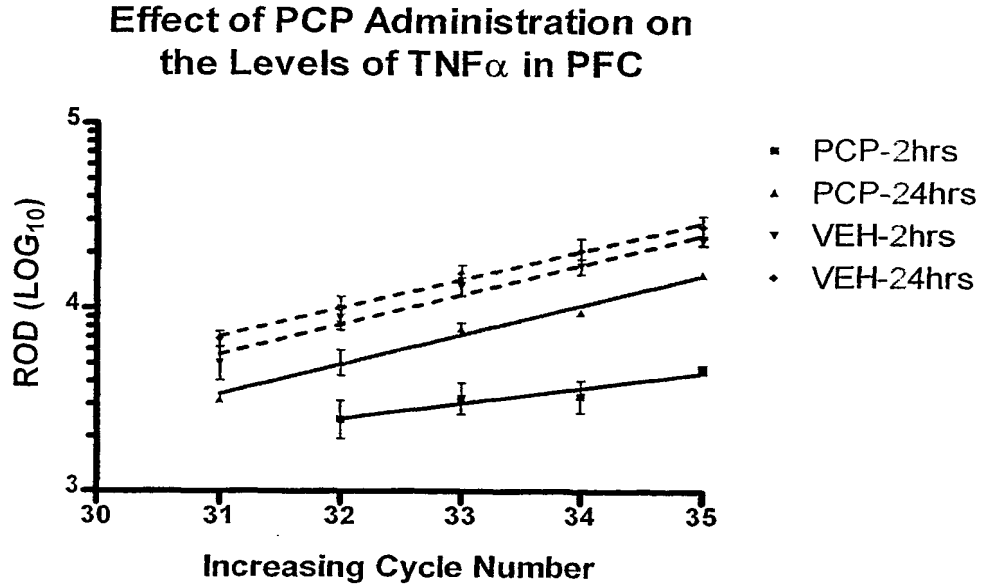



Figure 18

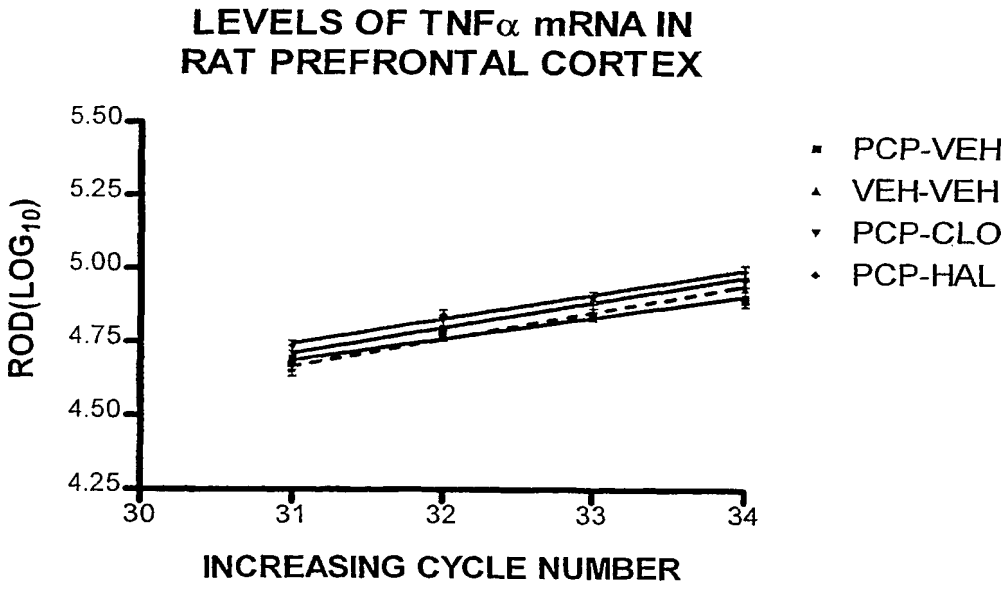
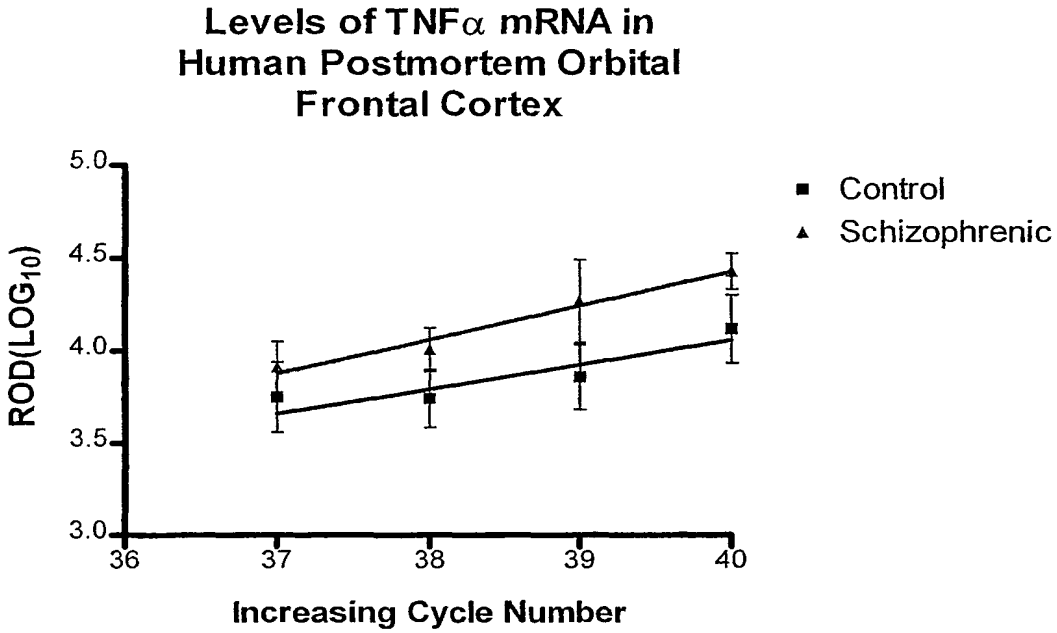


Figure 19


Levels of TNFα mRNA in Human Postmortem Orbital Frontal Cortex
5.0
4.5
4.0
3.5
3.0
ROD(LOG10)
36
37
38
39
40
Increasing Cycle Number
Control
Schizophrenic